Identifying drivers of glycaemic control in response to sulphonylurea treatment

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Abstract

Type 2 diabetes (T2DM) is a complex disease, brought about by the interaction of genetic and environmental factors. Coupled with a growing obesity problem, the disease has reached epidemic proportions globally. Several heterogeneous metabolic disorders including hyperglycaemia and impaired insulin secretion and/or action play a part in the progression of the disease. Large-scale cohort studies have identified several biomarkers for diabetes risk and for glycaemic control. The current study focused on the sulphonylurea drug class, which is associated with poor response rates and significant adverse events including an increased risk of hypoglycaemia. This study aimed to validate existing markers and to identify new biomarkers that may aid in patient stratification, especially in response to sulphonylurea therapy. 500 participants with T2DM were recruited from the Western Health and Social Care Trust and formed the basis of the DIASTRAT cohort. Clinical and anthropometric data suggest that those receiving sulphonylureas had significantly worse outcomes in terms of glycaemic control and BMI, especially when this was combined with an exogenous insulin reparation. Genetic studies confirmed a role for *ABCC8* and *KCNJ11* in sulphonylurea response from beta cell (β-cell) lines. Furthermore, several SNPs in *ABCC8*, *KCNJ11* and *HNF1*α were found to be significantly associated with glycemic control in participants receiving sulphonylurea therapy. A unique 75-protein signature was identified that correctly identifies those with T2DM to within 99.2% accuracy. Additionally, several of these proteins were found to correlate with glycaemic control in the DIASTRAT cohort. Overall, the work has identified several unique targets worthy of further validation in larger cohorts and secondary cohorts from other sites. Moreover, the work outlined in this thesis underlines the importance of considering both clinical and biological data for patient stratification.

Abbreviations

%	Percentage
ABC	ATP-binding cassette
ABCC8	ATP binding cassette subfamily C member 8
ADP	Adenosine monophosphate
ADOPT	A Diabetes Outcome Progression Trial
AGE	Advanced glycation end product
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BCA	Bicinchoninic
BMI	Body mass index
BSA	Bovine serum albumin
CaCl	Calcium chloride
cAMP	Cyclic adenosine monophosphate
cDNA	Complementary deoxyribonucleic acid
CFRD	Cystic Fibrosis Related Diabetes
CO_2	Carbon dioxide
CVD	Cardiovascular disease
ddH_2O	Distilled water
DFU	Diabetic foot ulcer
DIASTRAT	Stratified medicine optimizing treatment for Diabetes
DM	Diabetes Mellitus
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide
DPP4	Dipeptidyl peptidase 4
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
FBS	Fetal bovine serum

FFA	Free fatty acids
G	Gravity
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide 1
GLP-2	Glucagon-like peptide 2
GLUT2	Glucose-like transporter 2
GLUT4	Glucose-like transporter 4
GSIS	Glucose-stimulated insulin secretion
GWAS	Genome-wide association study
H	Hour(s)
HbA1c	Glycated haemoglobin
HDL	High-density lipoprotein
hESCs	Human embryonic stem cells
HNF1α	Hepatic Nuclear Factor 1 Alpha
INS	Insulin
K_{ATP}	ATP-sensitive K+
KCl	Potassium chloride
KCNJ11	potassium inwardly rectifying channel, subfamily J, member 11
KCNQ1	potassium voltage-gated channel subfamily Q member 1
KRB	Krebs Ringer Buffer
L	Litre(s)
LDL	Low-density lipoprotein
M	Molar(s)
Mg	Milligram(s)
$MgCl_2$	Magnesium chloride
MgSO4	Magnesium sulfate
min	Minute(s)
ml	Millilitre(s)
mM	Millimole(s) per litre
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NBD	Nuclear Binding Domain
NICE	The National Institute for Health and Care Excellence
nM	Nanomole(s) per litre
PBS	Phosphate-buffered saline
PEA	Protein Extension Assay
PFA	Paraformaldehyde
PKA	Protein kinase A
PP	Polypeptide
PPAR-γ	Peroxisome proliferator-activated receptor gamma
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
rpm	revolutions per minute
RT-PCR	Reverse-transcriptase polymerase chain reaction
SEM	Standard error of the mean
SGLT2	Sodium-glucose co-transporter-2
siRNA	Small interfering RNA
SNP	Single nucleotide polymorphism
SU	Sulphonylurea
SUR	Sulphonylurea receptor
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TCA	Tricarboxylic acid
TCF7L2	Transcription factor 7-like 2
tRNA	Transfer ribonucleic acid
UKPDS	UK Prospective Diabetes Study
UPR	Unfolded Protein Response
VDCC	Voltage-dependent calcium channels
WT	Wild type
µl	Microlitre(s)

µM	Micromole(s) per litre

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Chapter 1:

General introduction

1.1 The Endocrine pancreas

The human pancreas comprises both the exocrine and endocrine compartments; it plays a role in the digestion and glucose homeostasis through its production of a range of enzymes and hormones (Bramswig and Kaestner, 2012). The endocrine pancreas in particular, constitutes only 2% of the entire pancreas mass, with around 1 million islets of Langerhans (Bramswig and Kaestner, 2012). The islets are principally made up of five endocrine hormone producing cell types: Glucagon producing alpha-cells (α-cells), insulin producing beta-cells (β-cells), delta-cells (δ-cells) responsible for somatostatin secretion, the ghrelin releasing epsilon-cells (ε-cells) and pancreatic polypeptide (PP) producing cells (Mastracci and Sussel, 2012). In rodents, the β-cells form a core in the islet with α-cells and δ-cells around the periphery, while in the human pancreas the β-cells are dispersed randomly throughout the islet (Kim et al., 2009)

The endocrine pancreas receives between 5-15% of the organ's blood supply, from several arterioles per islet, which split into capillaries producing a spherical network (Ballian and Brunicardi, 2007). Pancreatic islet cells are innervated by the autonomic nervous system. Parasympathetic innervation potentiates insulin secretion during hyperglycaemic excursions, controls the cephalic phase of insulin secretion, when the body is preparing to use glucose most efficiently, and stimulates β-cell proliferation (Thorens, 2014). The sympathetic nervous system is however involved in the response to hypoglycaemia, and the formation of islet architecture and maturation during development (Thorens, 2014).

In type 2 diabetes there are some changes in the pancreas, particularly in relation to volume, which decreases, and the borders of the pancreas become more irregular when compared to a healthy pancreas (Al-Mrabeh et al., 2016). Type 2 diabetes is also marked by a decrease in functional β-

cell mass and volume, and gradual β-cell failure, comparative to the amount of insulin resistance observed (Cnop et al., 2005, Marselli et al., 2014).

1.2 Pancreatic Islet Hormones

The pancreas is responsible for the production and the secretion of three important hormones involved in glucose homeostasis and insulin secretion: insulin, glucagon and somatostatin (Chandra and Liddle, 2009). There are also PP secreting cells. Some studies have shown that PP is involved in regulating food intake and energy balance (Inui, 1999), however, an understanding of the functional of PP role has yet to be fully elucidated.

1.2.1 Insulin

Insulin is the peptide hormone released from the β-cells of the human endocrine pancreas; it is a 51-amino acid peptide with an A (21-amino acid) and B (30-amino acid) chain linked by 2 disulphide bonds (Wilcox, 2005). Insulin is synthesized by a precursor, proinsulin, the gene encoding this precursor is found on short arm of chromosome 11 (Schroder and Zuhlke, 1982). Proinsulin is synthesized, in the ribosomes of the rough endoplasmic reticulum from mRNA as pre-proinsulin, and then transported to the Golgi apparatus by secretory vesicles. Immature storage vesicles form here and proinsulin is converted to insulin and C-peptide by enzymes outside the Golgi apparatus (Wilcox, 2005). Insulin is stored in the lumen of the secretory vesicles and is released together with C-peptide and islet amyloid peptide in equal amounts (Fu et al., 2013). The insulin that is released goes to the portal venous system and 60% is removed during first pass through the liver (Wilcox, 2005). The liver is the principle site for the clearance of insulin, however the uptake and degradation of insulin occurs in all insulin sensitive

tissues (Duckworth et al., 1998). The kidney is another important site for insulin clearance and is responsible for removing around 50% of peripheral insulin, 50% of circulating proinsulin and 70% of C-peptide. This is achieved through glomerular filtration and reabsorption and degradation in the proximal tubule (Duckworth et al., 1998). All insulin sensitive cells are capable of removing and degrading insulin, the most notable after the liver and kidney is muscle (Duckworth et al., 1998).

Insulin acts as an anabolic agent; it encourages the storage and production of carbohydrates, lipids and protein and hinders their breakdown and discharge into circulation (Chang et al., 2004). Insulin encourages the production of glycogen and lipids in muscle cells, and subdues lipolysis and gluconeogenesis from muscle amino acids. In the muscle cells in particular, the entry of glucose allows storage and synthesis of glycogen, meaning carbohydrates will be used as the primary source of energy, instead of fatty acids (Wilcox, 2005). Insulin is vital for the transport of glucose into the cell. It acts to increase glucose uptake through enrichment of GLUT4 transporter proteins at the plasma membrane (Chang et al., 2004)
There are several factors that influence insulin synthesis and release, most notably, glucose. Insulin secretion can by impacted by changes in synthesis at the levels of gene transcription, translation and post-translational modification (Wilcox, 2005). The synthesis of insulin is also affected by amino acids, acetylcholine, pituitary adenylate cyclase-activating polypeptide, glucose dependent insulinotropic polypeptide, glucagon-like peptide-1 among others (Wilcox, 2005).

1.2.2 Glucagon

The α–cells of the endocrine pancreas are responsible for the secretion of the 29-amino acid peptide hormone, glucagon. This peptide has a potent stimulatory influence on hepatic glucose production, which works to increase plasma glucose levels (Lund et al., 2014). Glucagon is encoded by the preproglucagon gene (Taborsky, 2010). This gene has six exons, one encoding the glucagon precursor and two that encode the precursors for glucagon-like peptide (GLP)-1 and GLP-2. The products that come from this processing of proglucagon are tissue specific (Taborsky, 2010). When proglucagon is cleaved, only glucagon remains in its biologically active form, with the opposite being true for the GLP-1 and GLP-2 products. However, in the L-cells of the bowel, the opposite happens, with biologically active forms of GLP-1 and GLP-2 produced, and the inactive form of glucagon produced, also upon the cleavage of proglucagon (Taborsky, 2010).

Glucagon secretion is prompted during periods of low glucose; Ca^{2+} channels are opened following depolarization of the membrane, the subsequent influx of Ca^{2+} activates glucagon granule exocytosis from the cytoplasm (Briant et al., 2016). Glucagon acts to safeguard against a decrease in blood glucose (hypoglycaemia). This is achieved through its effects in the liver. Here, glucagon stimulates glycogenolysis and gluconeogenesis, causing a rapid increase in hepatic glucose output, and thus acting to restore normoglycaemia, (Briant et al., 2016). However, even a minor surge in glucose can induce long-lasting hyperglycaemia, for example a 10 pg/ml increase in glucagon can cause an upturn of hepatic glucose production by around 25% (Taborsky, 2010)

1.2.3 Somatostatin

Somatostatin is a polypeptide that is present in two main forms in humans, either as a 14-amino acid peptide (somatostatin 14) or the 28-amino acid form (somatostatin 28). Somatostatin 14-producing cells can be found in several peripheral organs – liver, lungs, immune system, urogenital tracts, kidneys, adrenals and the pancreas – while somatostatin 28 producing cells can be found along the gastrointestinal tract (Sliwinska-Mosson et al., 2014). This hormone has a regulatory role in the endocrine pancreas, where it is released from δ-cells following an increase in glucose concentrations. Somatostatin acts as an inhibitor of insulin, glucagon and pancreatic polypeptide release. However this is just one of many roles for somatostatin as it is also involved in inhibiting pain, the release of hypothalamic hormones, and in reducing gastrointestinal activity (Sliwinska-Mosson et al., 2014).

1.3 Regulators of pancreatic β-cell function

1.3.1 Amino acids

β-cell metabolism of different amino acids following ingestion and absorption of food can affect insulin secretion because of the consequent production of adenosine triphosphate (ATP) (Newsholme et al., 2005). The two most abundant amino acids found in circulation are L-glutamine and L-alanine, followed by branched-chain amino acids (Newsholme et al., 2005).

Amino acids play a role in the regulation of insulin secretion from the β-cell, which occurs via three distinct pathways (Keane and Newsholme, 2014). The first pathway involves the generation of ATP following tricarboxylic acid

metabolism. The second pathway involves the direct depolarization of the plasma membrane via interactions with amino acid transporters (Keane and Newsholme, 2014). The third pathway involves the depolarization of the plasma membrane, caused by the cotransport of Na^+ ions with the amino acid (Keane and Newsholme, 2014).

This suggests that amino acids can enhance glucose-stimulated insulin secretion, it has also been suggested that the effects of some amino acids can be damaging to β-cell function. For example, the metabolism of homocysteine can inhibit neuronal nitrous oxide synthase, this impacts the function of the β-cell because nitrous oxide synthase plays a key role in cell signaling and glucose uptake at low basal levels (Strain et al., 2004, Keane and Newsholme, 2014). This does not apply across the board for all amino acids however, with most having a positive effect on insulin release (Keane and Newsholme, 2014)

1.3.2 Incretin Hormones

There are two incretin hormones, both of which are found in the intestine. glucagon-like peptide 1 (GLP-1) is released from the L cells of the ileum, and glucose dependent insulinotropic polypeptide (GIP), is released from K cells in the duodenum (Kim and Egan, 2008). These hormones are released after ingestion of nutrients, with lipids and carbohydrates being the most effective macronutrient stimulants of incretin release (Phillips and Prins, 2011). Both of the incretin hormones function by increasing insulin secretion. However, GLP-1 also inhibits glucagon release, gastric emptying and works to reduce postprandial glucose excursions (Phillips and Prins, 2011).

G-protein coupled receptors for these two hormones are found on the pancreatic β-cell and when bound, they help promote the release of insulin

from the cells through the resulting production of 3',5'-cyclic adenosine monophosphate (cAMP). cAMP prompts a glucose-dependent increase in intracellular calcium, ultimately triggering insulin release (Kim and Egan, 2008, Phillips and Prins, 2011, Opinto et al., 2013). However, GLP-1 in particular has quite strong effects on the β-cell, as it also encourages the different stages of insulin biosynthesis and enhances β-cell function (Opinto et al., 2013). Furthermore, both have been shown to improve β-cell function in addition to lowering β-cell apoptosis (Phillips and Prins, 2011, Vasu et al., 2014). However, this incretin effect is reduced in both type 2 diabetes and obesity (Opinto et al., 2013)

GLP-1 and GIP are both rapidly degraded and inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4), causing a reduction in their insulinotropic effects (Yabe and Seino, 2011). DPP4 hydrolyzes the final alanine residue of each of the hormones, resulting in the production of the metabolites GIP(3-42) and GLP-1(9-37) these metabolites make up the most of the circulating forms of the incretin hormones (Opinto et al., 2013).

1.3.3 Glucose

Glucose, produced either through intestinal absorption, glycogenolysis or gluconeogenesis, is the primary regulator of insulin secretion from the β-cell (Roder et al., 2016). The pancreatic β-cell can act quickly to secrete insulin in response to changes in blood glucose levels. Insulin secretion is biphasic in nature with rapid release in the initial phase followed by a slower more sustained second phase of release (Rorsman et al., 2000, Roder et al., 2016). Glucose entry into the beta cell is facilitated by the membrane bound transporter GLUT2 (McClenaghan and Flatt, 1999). Glucose is then phosphorylated by glucokinase into glucose-6-phosphate, which is then metabolized to pyruvate and, following its oxidation through the

tricarboxylic (TCA) cycle, is converted to acetyl-CoA. This prompts closure of the ATP-sensitive potassium (K_{ATP}) channels due to the subsequent rise in the ATP:ADP ratio (McClenaghan and Flatt, 1999, Jensen et al., 2008). Closure of the K_{ATP} channel triggers membrane depolarization resulting in opening of voltage dependent Ca^{2+} channels (VDCCs). The ensuing rise in intracellular calcium triggers the exocytosis of insulin from secretory granules that fuse with the plasma membrane (McClenaghan and Flatt, 1999).

When hyperglycaemia persists for many months or years, it can trigger a series of damaging effects on the functions of the pancreatic β-cell, known as glucose desensitization (Brock et al., 2002). Persistent hyperglycaemia leads to glucotoxicity resulting in a reduction in insulin biosynthesis, increased apoptosis and diminished glucose-stimulated insulin secretion (Kosaka et al., 1980, Marshak et al., 1999, Maedler et al., 2001, Kong et al., 2015).

1.4 Diabetes mellitus

Diabetes Mellitus (DM) is a multifaceted, chronic metabolic disorder (Olokoba et al., 2012) that is ultimately characterised by hyperglycaemia resulting from insufficient insulin secretion or utilization (American Diabetes, 2014a).

DM is divided into the following main categories - Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) (American Diabetes, 2014b). However, there are other secondary forms of DM such as gestational DM(American Diabetes, 2010) and DM caused by other factors such as genetic deficiencies in β-cell function or insulin action, cystic fibrosis-related diabetes (CFRD) (Stecenko and Moran, 2010), also monogenic diabetes in

the form of MODY (mature onset diabetes of the young) (Anik et al., 2015) or even drug or chemical-induced diabetes seen in those receiving care for HIV/AIDS or post organ transplant (Fathallah et al., 2015).

T1DM occurs due to cell-mediated autoimmune destruction of the pancreatic β-cell (American Diabetes, 2014a) as a result of several genetic and environmental factors and the interactions between both (Wang et al., 2014). This destruction, which occurs due to development of autoreactive T cells capable of destroying pancreatic β-cells, results in decrease insulin secretory function (Atkinson, 2012). Of all diabetes patients around 10% are made up of T1DM patients (American Diabetes, 2014a). A feature of T1DM is the sustained need for exogenous insulin replacement (Atkinson et al., 2014). However, even with exogenous replacement, it does not necessarily mean that the metabolic regulation needed happens, thus management of T1DM can also include the use of insulin analogues or machine technology – insulin pumps being an example (Atkinson et al., 2014). When the necessary regulation does not occur through this form of treatment we see the rise in complications associated with T1DM. Complications can arise also due to a lack of adherence to medication, or from a non-response to treatment. Complications include, retinopathy, neuropathy, cardiovascular disease and hypoglycaemia (Atkinson et al., 2014). Other options for treatment include either whole pancreas, or islet-cell, transplantation. While outcomes for whole organ transplant are good, drawbacks remain due to the invasiveness of the procedure and the considerable perioperative risk (Ludwig et al., 2010). With islet-cell transplant, while preliminary reaction to transplantation is encouraging, typically after five years just 10% of patients stay free of exogenous insulin treatment (Atkinson et al., 2014).

T2DM arises when the insulin producing pancreatic β-cell can't produce enough insulin to deal with the rise in insensitivity (in certain tissues – muscle,

liver and fat) to the action of the insulin that has already been produced. Several factors play a role in this - age, obesity and lifestyle (Kahn, 2003, Scheen, 2003, Drong et al., 2012). Figure 1.1 details some contributing factors to T2DM. Around 90% of diabetes patients are made up of T2DM patients (American Diabetes, 2014a, Hex et al., 2012). The management of T2DM is complex and discussed later in section 1.5.2.

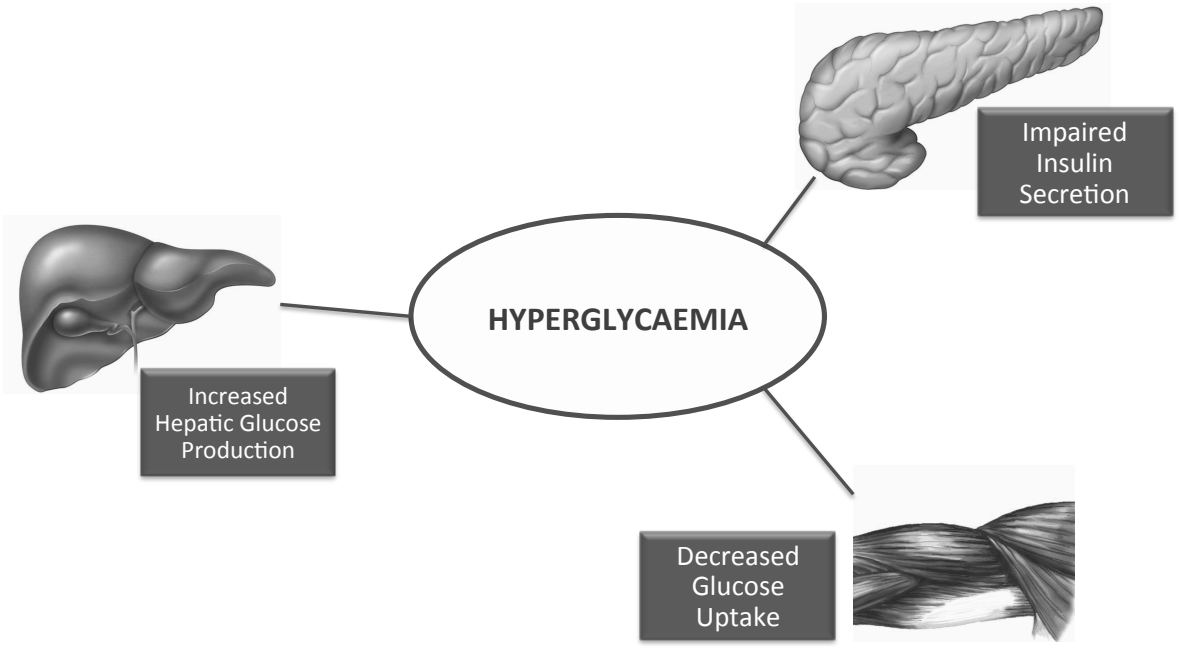


Figure 1.1: Factors contributing to a hyperglycaemic state in T2DM

This figure, adapted from De Fronzo, (2004), details a trio of metabolic defects that contribute to a hyperglycaemic state, which is a primary characteristic of T2DM.

Current estimations put the number of global diabetes patients at over 300 million and this number continues to rise (Alberti and Zimmet, 2014, Zimmet et al., 2014, Danaei et al., 2011). As of 2012, within the United Kingdom (UK) in particular there are and estimated 400,000 people with T1DM and 3,400,000 with T2DM (Hex et al., 2012). The current projection is that those numbers will rise to 650,000 and 5,600,000 people respectively by 2035/36 with around 150,000 newly diagnosed T1DM and T2DM patients every year

(Hex et al., 2012). The issue of not just treating diabetes but also the associated complications then worsens.

Some of the complications associated with T2DM in particular include cardiovascular disease, cardiac failure, stroke, kidney failure, retinopathy, angina, diabetic foot ulcers (DFU) and subsequent amputation. For example, cardiovascular disease (CVD) is the most common complication associated with T2DM, and is the primary cause of death in >60% of diabetes patients (Howard and Magee, 2000). DFUs arise in 15% of diabetes patients and are followed by diabetes-related lower-leg amputations in 84% of the cases (Brem and Tomic-Canic, 2007). The foremost cause of blindness in people of working age in the UK is diabetes (Diabetes UK, 2012, Arun et al., 2003). As well as this, the numbers affected by these unnecessary complications in England alone is rising. Between 2006 and 2010 retinopathy increased by 118%, stroke increased by 87%, kidney failure increased by 56%, cardiac failure by 43%, angina by 33% and amputations by 26% (Diabetes UK, 2012).

1.5 Type 2 diabetes mellitus

Glucose homoeostasis is controlled by a feedback loop, which is reliant upon interaction between the pancreatic β-cells and insulin sensitive tissues (Kahn et al., 1993). Insulin is released following the stimulation of the pancreatic β-cell. This in turn facilitates the acceptance of glucose, amino acids and fatty acids by these insulin sensitive tissues, after which the tissues then feed back information to the islet cells that they require insulin (Kahn et al., 2014). With patients who are obese, it is common to see insulin resistance (Kahn et al., 2014). This may result in the pancreatic β-cell upregulating insulin secretion as a way of trying to maintain a glucose

homeostasis. Conversely, if the pancreatic β-cell can't produce enough insulin, plasma concentrations of glucose will increase (Kahn et al., 2014).

1.5.1 Genetics of type 2 diabetes mellitus

Over the last decade, with increasing numbers of genome-wide association studies (GWAS), there is mounting evidence of the influential role that genetic factors play in individual risk to T2DM and in response to therapy (Fuchsberger et al., 2016). The hope has been that with the use of modern DNA sequencing techniques these studies will identify genetic variants that increase risk of disease, risk of adverse events in treatment, or helping to determine disease phenotype (McCarthy, 2017). However, the complex nature of T2DM – the impact of environmental factors, a broad-spectrum phenotype and the influence of common low-impact risk variants – highlights the difficulty with approaching the disease in this manner (McCarthy, 2017). Studies of this type have produced results though, for example ~90 loci have been detected in which variation will affect insulin release (Rutter, 2016).

Work has also shown how the rising global incidence of T2DM is inextricably linked to growing occurrence of obesity (Zimmet et al., 2001, McCarthy, 2010). Although, there are a lot of environmental factors involved in whether or not a person becomes obese and develops T2DM, it has been shown that there are individuals who are genetically predisposed to obesity (McCarthy, 2010). A variety of GWAS have been conducted in order to scrutinize a wide range of body mass index (BMI) values, in doing so they have catalogued around 30 loci that influenced BMI and the risk of obesity (McCarthy, 2010). In particular variation in the FTO (fat mass and obesity associated) gene that can predispose someone to diabetes by affecting their BMI (Frayling et al., 2007, McCarthy, 2010), 16% of adults that were

homozygous for the risk allele had a 1.67-fold increased odds of obesity as opposed to those who did not have the risk allele, this was witnessed from age 7 upward and an increase in fat mass was also seen (Frayling et al., 2007). The odds ratio (OR) is a way of quantifying how strongly the presence of one property is associated with the presence, or absence, of another (an OR = 1 suggests no additional risk of disease but a higher OR suggests a greater risk of disease). Visceral fat accumulation is known to have particularly negative health implications, with this in mind GWAS were conducted to analyse patterns of fat distribution resulting in the identification of 15 loci separate from those that influenced general adiposity, the loci have an impact on the wait to hip ratio, that is seen in women in particular more than men (Chambers et al., 2008, Lindgren et al., 2009, Heard-Costa et al., 2009, Heid et al., 2010, McCarthy, 2010).

Other large studies have shown a partial role for lower frequency variants in predisposition to T2DM, although not in as strong a fashion as common variants (Fuchsberger et al., 2016).

Further work, using CRISPR-based gene editing in human embryonic stem cells (hESCs) has also provided fresh insight into the function of a variety of genes previously identified in GWAS studies (Rutter, 2016, Zeng et al., 2016). This genome editing tool, which is seeing a swift rise in popularity, allows hESCs to be genetically manipulated allowing for those studying to understand the contribution of genes, and their variants, to a disease (Teo et al., 2015). This work has potentially identified *CDKAL1* – a gene that is important for first-phase insulin exocytosis in the β-cell, facilitating ATP generation, K_{ATP} channel closure and opening of Ca2+ channels as well as (Ohara-Imaizumi et al., 2010) – as a T2DM risk locus and a unique therapeutic target (previous work in mice and cells had failed to identify the importance of *CDKAL1*) (Zeng et al., 2016, Rutter, 2016).

1.5.2 Management of Type 2 diabetes mellitus

The approach for the management and treatment of T2DM has been designed by the National Institute for Health and Care Excellence (NICE), and is briefly summarised in Figure 1.2.

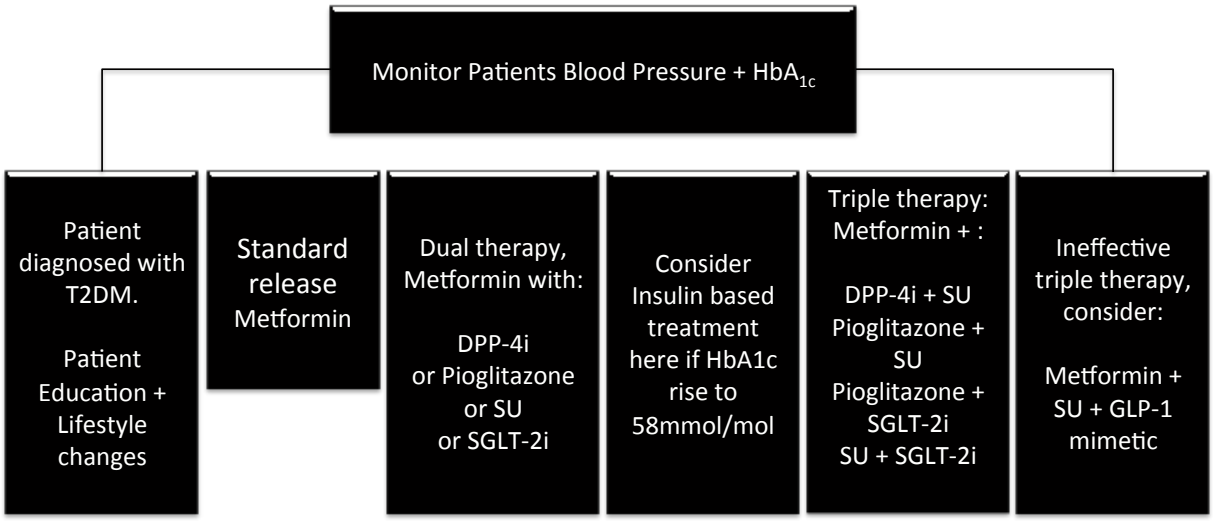


Figure 1.2: NICE Guidelines for treatment of T2DM

This figure, adapted from the NICE guideline 28, briefly describes the recommended treatment pathway in type 2 diabetes (SU, sulphonylurea,; DPP-4i, Dipeptidyl peptidase IV inhibitor,; SGLT-2i, sodium-glucose co-transporter 2 inhibitor,; GLP-1 glucagon like peptide 1) (National Institute for Health and Care Excellence (NICE), 2015).

The NICE guidelines have some key points to consider when making decision for treatment of T2DM, they are as follows.

Patient Education

Educational intervention ensures every patient and their carer receive a structured education about diabetes. This should take place at the time of diagnosis and should be reviewed and reinforced annually so that

education is a significant part of the patient's care (Sibal and Home, 2009). In 2015 NICE recommended that any educational programme for patients must have clear aims and objectives, be evidence-base, and meet the patient's individual needs. Trained educators should deliver a well-designed curriculum that is quality-assured and the outcomes of which should be regularly audited.

Dietary Advice

Nutritional management should provide the patient with dietary advice that is specific to them and should be updated regularly. Dietary advice should be combined with a personal diabetes management plan that should aim to increase the patient's physical activity and target a loss in body weight of 5-10% (Sibal and Home, 2009). However it has still been shown that exercise, even in the absence of weight loss, can be beneficial for patients (Ripsin et al., 2009) as it can help to decrease their blood pressure. Raised blood pressure in T2DM patients is associated with cardiovascular disease (CVD), eye and kidney microvascular complications (UKPDS Group, 1998b, Sibal and Home, 2009).

Blood Pressure Management

Medications should be added that helps to reduce blood pressure if the patient is unable to make changes in lifestyle, or if changes in lifestyle are not effective in reducing blood pressure to below 140/80 mmHg. This should be monitored every 1-2 months and treatment can be intensified if the patients blood pressure is not consistently below that level ((NICE), 2015).

Blood Glucose Management

Part of managing blood glucose is the setting of targets for haemoglobin A1c (HbA1c). Doctors should encourage patients to achieve and maintain

their target HbA1c, unless adverse effects, like hypoglycaemia, or their efforts to achieve the target, damage their quality of life (National Institute for Health and Care Excellence (NICE), 2015). Glycaemic control is defined as a HbA_{1c} of 48 mmol/mol, but where this is not realistically attainable, a target can be made with the patient so that reduction in HbA_{1c} still occurs. If the patient is unable to maintain a HbA_{1c} below 58 mmol/mol, drug treatment can be intensified and they should be advised to aim for a HbA1c of 53 mmol/mol. Some patients can be advised to self monitor blood glucose, but this shouldn't be done routinely, rather only in cases where the patient is prescribed insulin, where hypoglycaemic episodes are occurring, if the patient is at increased risk of hypoglycaemic episodes while driving or operating machinery, or if the patient is pregnant or plans to be ((NICE), 2015)

Drug Treatment

NICE recommendations on drug prescribing in T2DM are summarized in Figure 1.2. Standard release metformin is the first-line therapy for T2DM ((NICE), 2015). Metformin belongs to the biguanide class of drugs, which are and has been a drug of choice for use against T2DM for some time. They are an anti-hyperglycaemic class of drug that do not increase plasma insulin (Wrobel et al., 2017). Metformin is the only biguanide in clinical use. Metformin will diminish hepatic glucose production, decrease insulin resistance by allowing uptake and metabolism in skeletal muscle, lessen carbohydrate absorption, increase fatty acid oxidation, and decrease low-density-lipoprotein (LDL) and very-low-density-lipoprotein (VLDL) (Patane et al., 2000, Giannarelli et al., 2003, Natali and Ferrannini, 2006). The main mechanism of action comprises the triggering in hepatocytes of AMP-activated protein kinase (AMPK) that will impede the expression of genes essential for gluconeogenesis (Kim et al., 2008). The most common adverse

events seen with metformin use include anorexia, diarrhea and nausea (Bouchoucha et al., 2011), however there is significant interindividiual glycaemic response to metformin (Zhou et al., 2011).

Where metformin is contraindicated or not tolerated there are other options for initial treatment, either a dipeptidyl peptidase-4 (DPP-IV) inhibitor, or pioglitazone, or a sulphonylurea. DPP-4 inhibitors are a class of synthetic oral hypoglycaemic drugs, often referred to as gliptins (e.g. sitagliptin, vildagliptin). They act to depress blood glucose concentrations by potentiating endogenous incretins (Deacon, 2011). Pioglitazone is a thiazolidinedione, which decrease hepatic glucose output and increase glucose acceptance in the muscle by boosting the efficiency of endogenous insulin (Inzucchi, 2002). This is achieved when the drug binds to peroxisome proliferator-activated receptor-γ (PPARγ), which is complexed with retinoid X receptor. The exogenous agonists thiazolidinedione then cause this complex to bind to DNA, encouraging transcription of genes key to insulin signaling (Inzucchi, 2002). Severe hepatotoxicity has been encountered with two thiazolidinediones, ciglitazone and troglitazone (Patil et al., 2010); conversely this has not been seen in clinical trials of pioglitazone (Einhorn et al., 2000). Common adverse events seen with this particular thiazolidinedione include edema, heart failure, weight gain and fluid retention (Rizos et al., 2009). Other options include the insulin secretagogues, sulphonylureas. These drugs work by acting directly on the pancreatic β-cell to stimulate insulin secretion (Proks et al., 2002). They target the ATP-sensitive potassium (K_{ATP}) channel and subsequent inhibition of this channel triggers membrane depolarisation of the pancreatic β-cell. This prompts the opening of voltage-gated Ca^{2+} channels, which in turn provokes the entry of Ca^{2+} into the pancreatic β-cell, stimulating the exocytosis of insulin containing secretory granules (Proks et al., 2002). The most common adverse event observed with sulphonylureas is

hypoglycaemia. For some individuals, hypoglycaemia can be severe and persistent due to the duration of action of the drug, particularly with first generation sulphonylureas like tolbutamide and chlorpropamide (Aquilante, 2010).

In some cases it is necessary to combine therapies. However, if triple therapy with metformin and two other oral drugs is not effective, or is not being tolerated, then NICE guidelines suggest adding a glucagon-like peptide-1 (GLP-1) mimetic to metformin and a sulphonylurea ((NICE), 2015). The GLP-1 mimetic Exenatide works by mimicking the effect of GLP-1, but is markedly more stable, and longer acting (Mann and Raskin, 2014). It can lower postprandial blood glucose concentrations by stimulating insulin secretion, subduing glucagon secretion and impeding gastric emptying (Mann and Raskin, 2014). It has been known to cause hypoglycaemia, and in rare cases even pancreatitis (Garg et al., 2010, Mann and Raskin, 2014). GLP-1 mimetics should only continue to be prescribed if the patient responds within 6 months. The response target set by NICE includes an 11 mmol/mol reduction in HbA1c and weight loss of 3% of initial body weight ((NICE), 2015).

T2DM patients who have had combined therapy, but remain significantly hyperglycaemic are considered for exogenous insulin therapy (Sibal and Home, 2009). Modern insulin therapy uses almost completely human insulin, produced using recombinant DNA technology. Different formulations of insulin that are used also have varying time of peak effect and duration of action (Swinnen et al., 2009). Eventually nearly all T2DM patients will require insulin therapy (Niswender, 2009). Insulin works by binding to the insulin receptor, a large transmembrane glycoprotein complex from the tyrosine kinase-linked type 3 receptor superfamily, located on the plasma membrane of target cells (Wilcox, 2005, Lee and White, 2004). Binding to

the receptor allows adenosine triphosphate (ATP) to bind to the intracellular component of the receptor, causing phosphorylation of the receptor through tyrosine kinase activity (Wilcox, 2005). The result of this is the tyrosine phosphorylation of intracellular substrate proteins – insulin responsive substrates (IRS), which then bind other signaling molecules and promote further cellular actions of insulin (Wilcox, 2005). The internalised insulin is degraded in lysosomes, however the receptors are recycled to the plasma membrane (Lee and White, 2004). Some patients may continue to receive metformin while using insulin if there are not contraindications or intolerance ((NICE), 2015).

1.6 Response to treatment

It is clear that in order for stratification of patients to take place, there needs to be an unambiguous definition of response to treatment. The marker used to determine the glycaemic control of a T2DM patient is glycated haemoglobin (HbA_{1c}), which provides information on glycaemic control for the previous two to three months (Sherwani et al., 2016). Haemoglobin undergoes regular glycation, as part of normal physiological function and when this occurs, the most abundant fraction post-glycation is HbA_{1c} (Sherwani et al., 2016). The amount of circulating HbA_{1c} is directly proportional to the amount of circulating blood glucose (Sherwani et al., 2016). The erythrocytes, which are rich in haemoglobin, typically have a two to three month lifespan, explaining why the HbA_{1c} measurement gives such a long view of glycaemic control; typically this measurement is taken every three to six months in those with T2DM ((NICE), 2015, Sherwani et al., 2016). It is common to see response to treatment in T2DM defined in one of two ways: either as a magnitude of HbA1c change (DeFronzo et al., 2010), or a target HbA_{1c} (Esposito et al., 2012). Some studies have shown that baseline HbA_{1c} plays an important role in determining whether or not a patient

reaches their target HbA1c, or attains a desired magnitude of change. Essentially, those starting with a higher baseline HbA_{1c} will see larger reductions, but they are also less likely to reach a 53 mmol/mol (6.9 %) target (DeFronzo et al., 2010, Esposito et al., 2012). This must be considered when assessing clinical efficacy of treatments. However there are two ways of potentially overcoming the effect caused by baseline HbA_{1c}. The first involves altering the definition of response. Jones et al. (2014), showed that when different definitions of response are used, different groups of responders are identified. By way of example, a lower baseline HbA_{1c} is associated with good response, when the definition of response is based on reaching target HbA_{1c}. This group proposed a novel definition that combined both definitions, a change of < -21 mmol/mol with a HbA_{1c} achieved of < 62 mmol/mol, and with the use of this novel definition were able to create a group of responders that did not vary as a result of baseline HbA_{1c} (Jones et al., 2014). The second option has again been proposed by Jones et al. (2016), who after again demonstrating that baseline HbA_{1c} is a major predictor of response, determined that adjusting data for baseline HbA_{1c} was a successful method that can be used to reduce bias in studies where the aim is to identify a predictor of response, other than baseline HbA_{1c}.

1.7 Sulphonylureas: Response and adverse events

As outlined above and in Figure 1.3, Sulphonylurea acts on the pancreatic β-cell to encourage insulin secretion.

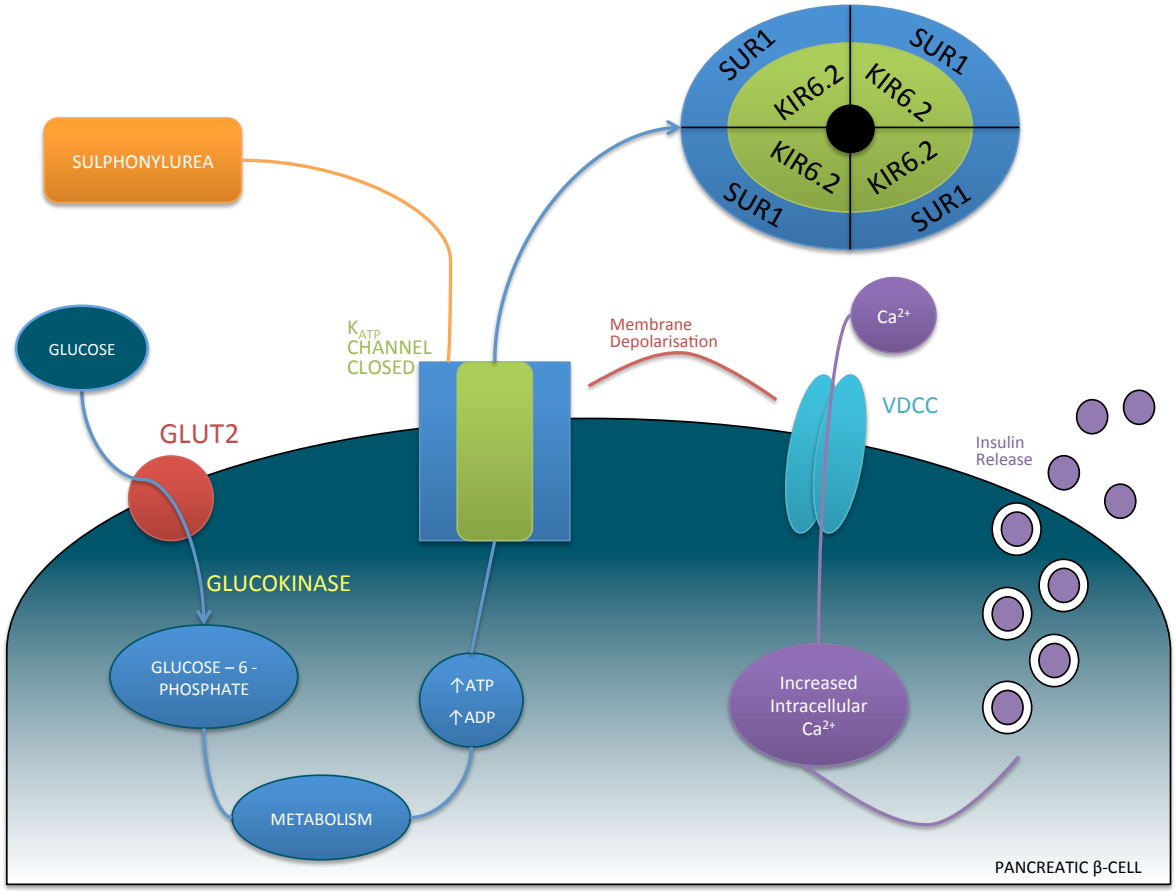


Figure 1.3: Insulin release following sulphonylurea exposure

This figure shows sulphonylurea binding to the SUR1 receptor on the K_{ATP} channel and the subsequent downstream effects that lead to insulin release from the pancreatic β-cell. Briefly, sulphonylurea binding causes closure of the K_{ATP} channel, membrane depolarisation, opening of voltage-dependent calcium channels, an influx of calcium into the β-cell, and as a final consequences, insulin exocytosis. (GLUT2, glucose transporter 2,; ATP, adenosine triphosphate,; ADP, adenosine diphosphate,; K_{ATP}, ATP sensitive potassium channel,; SUR1, sulphonylurea receptor 1,; Kir6.2, inwardly rectifying potassium channel,; VDCC, voltage dependent calcium channel)

The normal activity of the β-cell K_{ATP} channel is governed by intracellular adenosine nucleotide; ATP causes an inhibitory effect on channel activity and a stimulatory effect caused by Mg-nucleotides (MgATP or MgADP)

(Proks et al., 2010). The K_{ATP} channel is a hetero-octameric complex comprising two types of protein subunit – Kir6.2, and sulphonylurea receptor 1 (SUR1) (Ashcroft and Gribble, 1999, Proks et al., 2002). Kir6.2 is a member of the family of inwardly rectifying K^+ (Kir) channels, it assembles as a tetramer forming the channel pore (Proks et al., 2002). The Kir6.2 subunits have a binding site for adenine nucleotides; the pore will close when either ATP or ADP binds to one or more of these sites (Proks et al., 2010). The Kir6.2 subunits are complexed with SUR1 subunits, each containing ER retention signals, which will inhibit their movement to the plasma membrane when the partner subunit is absent (Proks et al., 2010). SUR1 is part of the ABC transporter family, with 17 transmembrane helices (TMs). The TMs are organized as a single group of 5 TMs, with two repeats of 6 TMs, followed by a large cytosolic loop with consensus sequences for nucleotide binding and hydrolysis (Proks et al., 2002). The Kir6.2 pore achieves sensitivity to the stimulatory effects of nucleotides, and activation or inhibition by therapeutic drugs from SUR1 (Nichols et al., 1996, Gribble et al., 1997a, Gribble et al., 1998a, Proks et al., 2010). SUR1 has two nucleotide-binding domains (NBDs) that take part in binding and hydrolysis of Mg-nucleotides (Proks et al., 2010). The NBDs combine in a head-to-tail fashion, the Walker motifs of one interact with the linker domain of the other and form 2 composite ATP-binding sites (Proks et al., 2010). Although the events taking place at site 1 are not clear, it is known that the channel can be activated whenever MgADP occupies site 2 (Zingman et al., 2001, Proks et al., 2010).

There are two groups of K_{ATP} channel activity inhibitors – one group that includes Imidazolines and antimalarials which bind directly to Kir6.2 to block K_{ATP} channel activity, or the other group which bind with high affinity to SUR causing closure of K channels (for example sulphonylureas or benzamido derivatives) (Proks and Ashcroft, 1997, Gribble et al., 1997b, Mukai et al.,

1998, Gribble et al., 1998b, Gribble and Ashcroft, 1999, Gribble et al., 2000, Proks et al., 2002).

There are three main types of sulphonylurea receptor subunit, SUR1 - shapes part of the K_{ATP} channel in endocrine cells and brain, SUR2A – is found in heart and skeletal muscle, SUR2B encompasses the smooth muscle K_{ATP} channel (Proks et al., 2014). Sulphonylureas will bind to their particular receptor with high affinity prompting the pore to shut (Proks et al., 2014). This inhibition though, is incomplete and reaches a maximum of around 50-80%, this is as a result of K_{ATP} channels with bound sulphonylurea still being open to some extent (Gribble et al., 1997b, Barrett-Jolley and Davies, 1997, Proks et al., 2014). Therefore, sulphonylureas are considered partial antagonists of the K_{ATP} channel (Proks et al., 2014).

The position of the binding site for sulphonylureas on the K_{ATP} channel has yet to be fully elucidated. However, some evidence suggests that residues within the intracellular loop between transmembrane domains 5 and 6, and a residue in the intracellular loop between transmembrane domains 15 and 16, play a role (Ashfield et al., 1999, Vila-Carriles et al., 2007, Proks et al., 2014). Sulphonylureas can also interact with Mg-nucleotides to regulate K_{ATP} channel activity (Proks et al., 2014). An example being the obstructive effect that MgATP and MgADP have on glibenclamide's ability to bind to either SUR1 or SUR2 (Hambrock et al., 2002). Equally, MgADP can enrich the sulphonylurea inhibition of β-cell K_{ATP} channels (Proks et al., 2002).

A difficulty with sulphonylurea treatment is interindividiual variation in response to the drug. While different studies use different definitions of failure to respond, 10-20% of patients will suffer from primary sulphonylurea failure. That is, upon initial sulphonylureas administration, they will have less than a 20-mg/dl reduction in fasting plasma glucose (DeFronzo, 1999,

Aquilante, 2010). Whereas, in the region of 50-60% of patients will ultimately fail to reach the required glycaemic control following a primary decrease in fasting plasma glucose of greater than 30mg/dl (DeFronzo, 1999, Aquilante, 2010). There is no clear reason why the failure rate is so high. Secondary sulphonylurea failure is also an issue in around 5-7% of patients who have a good response to the drug class initially (DeFronzo, 1999, Aquilante, 2010). This varying response has also been replicated in large-scale randomized trials; in particular the A Diabetes Outcome Progression Trial (ADOPT) revealed that sulphonylurea monotherapy failure was 34%, contrasted with 15% for rosiglitazone and 21% for metformin (Kahn et al., 2006, Aquilante, 2010). In addition to this variation in the successfulness of sulphonylurea treatment, variation is also seen in the adverse events experienced as a result of sulphonylurea treatment. Approximately, 31% of patients suffer mild hypoglycaemia in the first year of glibenclamide treatment (UKPDS Group, 1998a). However, the frequency of severe hypoglycaemia is estimated at 1% per year (UKPDS Group, 1998a). The UKPDS study defined a mild hypoglycaemic event as one where the patient could treat the event unaided, and severe was defined as when the patient needed help from a third party or medical assistance. There are clinical factors involved in the failure of sulphonylurea treatment including deteriorating β-cell function, long-standing diabetes, elevated baseline glucose levels and an elevated degree of insulin resistance (Aquilante, 2010). The same could also be said when it comes to sulphonylurea induced hypoglycaemia, there are clinical factors involved like - the duration of sulfonylurea action, mild baseline hyperglycemia, irregular eating patterns, excessive alcohol intake and age (Aquilante, 2010). However, these clinical factors are not the only factors at play in variation to sulphonylurea response, disposition and side effects (Aquilante, 2010). There is evidence suggesting that genetic polymorphisms are involved in the interindividual variation (Aquilante, 2010).

Genes involved in response to sulphonylureas, and risk to T2DM, include the ATP Binding site cassette, subfamily C, member 8 (ABCC8). ABCC8 encodes for the sulphonylurea receptor 1 (SUR1) protein, it is positioned at 11p15.1 and contains >100kb of genomic DNA (Kapoor, 2010, Haghverdizadeh et al., 2014). The ABCC8 gene contains 39 exons, and some 2318 common and rare SNPs (Haghverdizadeh et al., 2014). From this large number some SNPs have received more notice in the literature, 9 are present in coding regions, the rest in non-coding (Haghverdizadeh et al., 2014).

Another important gene to note is the potassium inwardly rectifying channel, subfamily J, member 11 (KCNJ11) gene, which encodes for Kir6.2 an essential part of the K_{ATP} channel which is found in pancreatic β-cells, it is located at 11p15.1 (Qin et al., 2013). Polymorphisms of this gene have been shown to predispose individuals to T2DM (Gloyn et al., 2005) as well as affecting the therapeutic response to sulphonylurea treatment (Aquilante, 2010, Javorsky et al., 2012). Additionally, there is hepatocyte nuclear factor-1α (HNF-1α), a gene located at 12q24.2, the protein encoded by this gene is HNF-1α transcription factor, involved in the regulation of the tissue specific expression of different genes in pancreatic islet, the liver and the kidney (Hansen et al., 1997, McDonald et al., 2012). This gene has been identified as having a part to play in the pathogenesis of T2DM (Giuffrida et al., 2009), however it is more commonly known as mutations in this gene are the most common cause of Maturity Onset Diabetes of the Young (MODY) in the UK (McDonald et al., 2012). Finally, the potassium voltage-gated channel, KQT-like subfamily, member 1 (KCNQ1) gene encodes for the pore-forming subunit of a voltage gated potassium channel (KvLQT1) that can be found in the pancreatic β-cell (Travers et al., 2013, Schroner et al., 2011). The gene is located at 11p15.5 and it has been acknowledged as being a risk gene associated with T2DM (Kong et al., 2009, Travers et al., 2013), as well as a

gene that is involved in the therapeutic response to sulphonylureas (Schroner et al., 2011).

1.8 Stratification: Patient Benefit and Healthcare Economics

Stratified medicine is defined as the division of patients into groups based on either their risk of disease or their response to a given treatment (Lonergan et al., 2017). The intention then is that drug dosages can be refined or more suitable treatments identified for specific patient groups. This decision is brought about through the use of biomarkers and different measures of the advantages and the cost for treatment of each group (Lonergan et al., 2017). The benefit then of stratifying treatment comes from the patient receiving an improved treatment at an earlier stage and a reduction in waste for the health service provider (Lonergan et al., 2017).

Placing that knowledge within the context of the cost of treating T2DM shows the importance of stratification in the wider picture. With the large numbers of patients with T2DM, there is a sizeable cost burden placed on the health service. As of 2010/11 the burden of direct patient care was estimated at £9.8bn and indirect costs associated with diabetes estimated at £13.9bn (Hex et al., 2012). The direct costs have been divided between treatment/intervention and complications/adverse events, with the former costing over £2bn and the latter costing £7.7bn (Hex et al., 2012). It is clear from these figures that the liability, in terms of costs, to the NHS is significant, accounting for approximately 10% of total NHS resource expenditure, a number which is again expected to rise (Hex et al., 2012).

It is well understood that HbA_{1C} is an important indicator of long-term glycaemic control (Sherwani et al., 2016) and that raised HbA_{1c} is greatly

associated with an increased risk of T2DM associated complications (principally CVD) and mortality (Sherwani et al., 2016). Improving glycaemic control has a positive impact on risk to further complications (UKPDS Group, 1998a). It has been shown that a HbA_{1c} of 53 mmol/mol (7.0%) instead of 63 mmol/mol (7.9%), for example, sees a 25% reduction in microvascular complications (UKPDS Group, 1998a, Baxter et al., 2016).

In the context of stratifying treatment, initiation of the correct therapy at the earliest possible opportunity will not only improve outcomes for the patients but also have a great impact on cost burden to the NHS. Recent work has shown that getting the right treatment sooner to diabetes patients could save the NHS around £340 million in the first five years and around £5.5 billion after 25 years (Baxter et al., 2016). Most of these savings are as a result of the reduction in microvascular complications. With T2DM in particular, the savings are seen through the reduction in foot ulcers, amputations and neuropathy (Baxter et al., 2016).

1.9 Aims of this thesis

Large-scale cohort studies have identified several biomarkers for T2DM risk and for sulphonylurea response. This study aimed to validate existing markers and to identify new biomarkers that may aid in patient stratification, especially in response to sulphonylurea therapy. Specific objectives include:

1. Identify and validate existing markers of sulphonylurea response in two pancreatic β-cell lines.
2. Establish a biobank of 500 T2DM patients (DIASTRAT cohort) with associated clinical, anthropometric, and biochemical data.

3. Conduct proteomic screens for novel inflammatory or metabolic markers associated with glycaemic control and sulphonylurea response in the DIASTRAT cohort
4. Assess the prevalence of potentially important SNPs among the DIASTRAT cohort and assess the relationship with glycaemic control and sulphonylurea response.

Chapter 2

Materials and methods

2.1 Materials

Reagents of analytical grade and deionized water (obtained from Elga, High Wycombe, UK) were used. Sources of each reagent are as follows: ***Sigma Aldrich (Irvine, UK):*** Dimethylsulfoxide (DMSO) "for molecular biology", PAP Pen for immunostaining, 4-didget hand-held tally counter, Poly-prep slides – poly-L-lysine coated glass slides, Sodium chloride (NaCl), Potassium chloride (KCl), Calcium chloride ($CaCl_2$), Magnesium sulphide ($MgSO_4.7H_20$), Magnesium chloride hexahydrate BioXtra ($MgCl_2.6H_20$), Sodium phosphate dibasic BioXtra ($Na_2HPO_2H_20$), Monobasic potassium phosphate (KH_2PO_4), D-Glucose, Bovine Serum Albumin (BSA), Tween 20, ethanol, Triton X-100, , beta-actin monoclonal antibody mouse, C,N-diphenyl-N'-4,5-dimethyl thiazol 2 yl tetrazolium bromide (MTT), Goat serum, Hydrochloric acid (HCl) and paraformaldehyde (PFA). ***Thermo Fisher Scientific (UK):*** Pierce protease and phosphatase inhibitor mini tablets, RIPA lysis and extraction buffer, Platinum TAQ DNA polymerase, Trackit 100 bp DNA ladder, 100 MM DNTP set, Roswell Park Memorial Institute (RPMI) 1640 cell culture media with L-glutamine and supplemented with Penicillin/Streptomycin and Fetal Bovine Serum (FBS) South American, Dulbeccos Modified Eagle Medium (DMEM) supplemented with Penicillin/Streptomycin, Fetal Bovine Serum (FBS), 2-β Mercaptoethanol and sodium pyruvate, Trypsin 0.5% EDTA, Agarose, Gel Red Stain loading dye, Trackit Cyan/Yellow loading buffer and black clear-bottomed 96 well plate for fluorescence. ***Roche Diagnostics (West Sussex, UK):*** Lightcycler 480 Probes Master Mix, Lightcycler plates 480 Multiwell plate, Transcriptor First Stand cDNA synthesis kit, Transcriptor Reverse Transcriptase, Protector RNase Inhibitor, Deoxy-NTP set. ***Abcam (Cambridge, UK): .ALPCO/Stratech Scientific (Newmarket, UK):*** Rat Insulin ELISA kit. ***Santa Cruz Biotech (Dallas, US):***. ***Mercodia (Uppsala, Sweden):*** Ultrasensitive Mouse Insulin ELISA kit and Insulin ELISA Mouse kit. ***Merck Millipore (Watford, UK):***. ***Qiagen (Manchester, UK):*** RNeasy Mini Kit (250) and Premix FlexiTube siRNA – predesigned siRNA directed against ABCC8, KCNJ11, KCNQ1 and HNF1α

PHE Culture Collection (Salisbury, UK): General Cell Collection: BRIN-BD11. ***Oxoid (Basingstoke, UK):*** Phosphate buffered saline (PBS) solution tablets.

2.2 Methods

2.2.1 Culture of β-cell lines

The pancreatic β-cell line BRIN-BD11 is a glucose responsive insulin-secreting cell line of rat origin. The cell line was created at Ulster University through the electrofusion of primary rat islets and the RINm5F cell line. Prior reports show that BRIN-BD11 cells are useful for analyzing the molecular mechanisms by which β-cells regulate insulin secretion (McClenaghan et al., 1996). The pancreatic β-cell line MIN6 is insulinoma-derived from transgenic mice containing the human insulin promoter gene connected to the SV40 T-antigen hybrid gene (Miyazaki et al., 1990, Ishihara et al., 1993). These cells have levels of glucose-induced insulin secretion that are similar to cultured mouse islet cells and are regarded as a valuable tool in the analysis of the molecular mechanisms by which β-cells regulate insulin secretion in response to extracellular glucose (Miyazaki et al., 1990). Both cell lines grow as adherent monolayers with epithelial-like appearance as shown in Figure 2.1, and were chosen due to their reported expression of genes of interest, which is discussed later. While both cell lines produce insulin in response to glucose and are recognised as good models for studying pancreatic β-cells, they of course are not the same as a primary human pancreatic β-cell, a caveat that is important to remember when interpreting any results seen following their use in experiments.

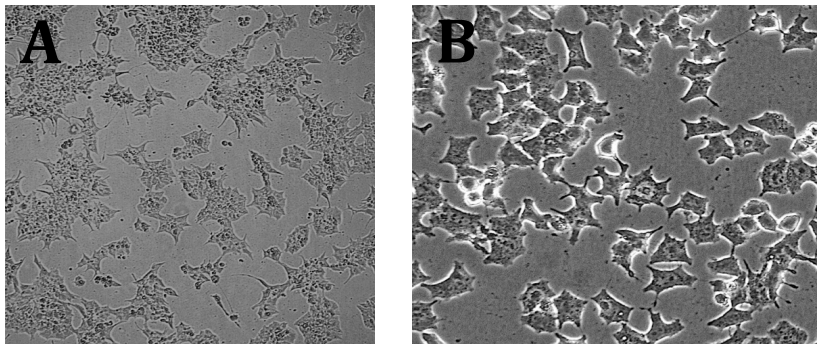


Figure 2.1: Pancreatic β-cell lines in culture.

(A) x4 magnification of MIN6 cell monolayers; (B) x20 magnification of BRIN-BD11 cell monolayers.

All cells were stored at the NI Centre for Stratified Medicine, Ulster University, in 1.5 ml vials (Sterilin Ltd, Hounslow, UK) containing freezing media with a final composition of 10 % DMSO, 10 % tissue culture medium, and 80 % foetal bovine serum (FBS). The cells were stored at -20 °C for 4 h, before being moved for further storage at -80 °C. Cells were removed from the freezer and thawed in a water bath maintained at 37 °C. The contents of the vials were suspended in 5 ml of warmed tissue culture medium and centrifuged for 5 min at 900 rpm (10,000 g). The cell pellet was re-suspended in fresh culture medium to give a single cell suspension, which was placed in a tissue culture flask and stored in an incubator at 37 °C, at an atmosphere of 5% CO_2 and 95% air. MIN6 cells were maintained in 75 cm^2 tissue culture flasks containing DMEM tissue culture medium with 4.5 g/L D-Glucose, 580 mg/L L-Glutamine, and supplemented with 10% (v/v) heat inactivated FBS, Penicillin/Streptomycin (5000 IU/l), 1x Sodium Pyruvate and 0.05 mM β-mercaptoethanol. BRIN-BD11 cells were maintained in 175 cm^2 tissue culture flasks containing RPMI tissue culture medium supplemented with 10% (v/v) FBS and Penicillin Streptomycin (5000 IU/l).

The following procedure was used to routinely passage the cells: culture medium was aspirated from the flask and cells washed twice with 5 ml of phosphate buffered saline solution (PBS). A total of 2 ml of trypsin-EGTA (1x) was used to detach cells from the tissue culture flask. After addition

of trypsin, cells were returned to the incubator at 37 °C, at an atmosphere of 5% CO_2 and 95% air, for 3-5 min. The flask was gently tapped to aid dissociation and cells were then re-suspended in 3 ml of tissue culture medium and transferred to a polypropylene Sterilin tube (Sterilin Ltd, Hounslow, UK). Cells were then pelleted by centrifugation at 900 rpm (10,000 g) for 5 min. The supernatant was discarded and fresh culture medium was added to the cells to create a single cell suspension for experimental use. Aliquots of the cell suspension were counted using a Neubauer haemocytometer (Scientific Supplies Co., UK). Cells were returned to culture and allowed to attach overnight as monolayers or utilized for experiments.

2.2.2 Induction of insulin secretion

Acute, static, 20 min incubations with sulphonylureas were conducted to assess insulin secretion from the BRIN-BD11 and MIN6 cell lines. A total of 1.5 x 10^5 BRIN-BD11 cells/well, or 1 x 10^5 MIN6 cells/well, were seeded in 24 well plates and allowed to attach overnight. Following this an Acute insulin response test designed to mimic first phase insulin secretion was performed. Firstly, media was removed from each well and cells were treated with Krebs-Ringer Bicarbonate (KRB) Buffer (115 mM NaCl, 4.7 mM KCl, 1.28 mM $CaCl_2$ 1.2 mM $MgSO_4$, 0.1% BSA, pH 7.20) supplemented with 1.1 mM glucose. The plate was then returned to the incubator at 37 °C, at an atmosphere of 5% CO_2 and 95% air for 40 min, after which the 1.1 mM glucose was removed and replaced with 1 ml of test solution (KRB buffer supplemented with 5.6 mM glucose ± 200 μM Glibenclamide or Tolbutamide). The plate was returned to the incubator for a further 20 min. Following incubation the supernatant was transferred to a fresh eppendorf and stored at -80 °C until needed for further experimentation.

2.2.3 Insulin ELISA for analysis of cell culture supernatants

Insulin release from BRIN-BD11 or MIN6 cells into the surrounding culture medium in response to sulphonylurea treatment was assessed using rat (ALPCO, Salem, NH, USA), or mouse (Mercodia, UK) specific insulin ELISA kits according to the manufacturers' instructions.

In brief, assessment of rat insulin concentrations (ALPCO, Salem, NH, USA) involved adding 10 µl of each calibrator, sample and control to wells of a supplied 96 well plate treated with primary antibody against insulin. Following the addition of samples, 75 µl of enzyme conjugate was added; the plate was covered in foil and placed on a plate shaker at 900 rpm for 2 h at room temperature. Enzyme conjugate was diluted 1:10 with conjugate stock provided in the kit. Each well was then washed 6 times with 350 µl of wash buffer. Working strength wash buffer was prepared by diluting wash buffer concentrate that had been supplied with 20 parts distilled water. Substrate TMB (100 µl) was added to each well, and the plate further incubated at room temperature for 15 min. Finally, 100 µl of stop solution was added and absorbance was read immediately at 450 nm using an Epoch Microplate Spectrophotometer (BioTek, UK). A typical standard curve is illustrated in Figure 2.2.

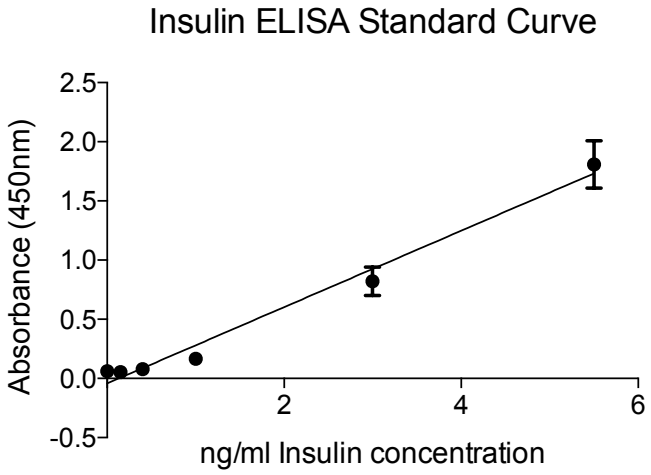


Figure 2.2: Typical rat Insulin ELISA standard curve

An insulin standard curve ranging from 0.15 – 5.5 ng/ml was created with the standards provided.

Assessment of mouse insulin concentrations was conducted by adding 25 µl of each calibrator, sample and control to the appropriate well of a supplied 96 well plate coated with primary antibody against insulin. Following the addition of samples, 100 µl of enzyme conjugate was added; the plate was covered in foil and placed on a plate shaker at 900 rpm for 2 h at room temperature. Each well was then washed 6 times with 350 µl of wash buffer. Working strength wash buffer was prepared by diluting wash buffer concentrate that had been supplied with 20 parts distilled water. Substrate TMB (200 µl) was added to each well, and the plate further incubated at room temperature for 15 min. Finally, 50 µl of stop solution was added to each well and absorbance was read immediately at 450 nm using an Epoch Microplate Spectrophotometer (BioTek, UK). A typical standard curve is illustrated in Figure 2.3.

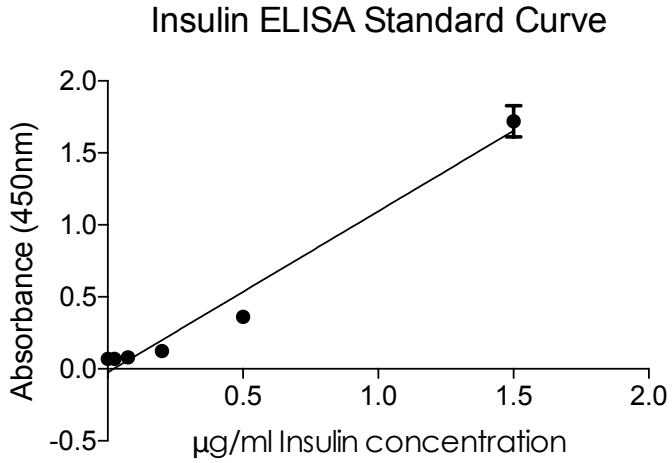


Figure 2.3: Typical Mouse Insulin ELISA standard curve

An insulin standard curve ranging between 0 - 1.5 μg/ml was created with the standards provided.

2.2.4 Extraction and quantification of RNA

Total RNA was extracted from BRIN-BD11 and MIN6 cells using the QIAGEN RNeasy® Mini Kit (QIAGEN, West Sussex, UK). This kit allows up to 100 µg of RNA longer than 200 bases to bind to the RNeasy silica membrane using a specialized high salt buffer system. Disruption of cell

pellets was achieved through the addition of 350 µl of Buffer RLT. A total of 350 µl of 70 % molecular-grade ethanol (Sigma Aldrich, UK) was then added to the lysates and mixed, providing a suitable binding condition for the silica membrane. The lysates were pipetted onto an RNeasy Mini spin column, placed in a 2 ml collection tube and centrifuged at 10,000 rpm (8,000 g) for 15 s in a microcentrifuge. Buffer RW1 (700 µl) was added, and samples were further centrifuged to clean the spin column membranes for 15 s at 10,000 rpm (8,000 g). Buffer RPE (500 µl) was added to the spin columns, which were centrifuged again for 15 s at 10,000 rpm (8,000 g). This step was then repeated with an additional minute of centrifugation at 10,000 rpm (8,000 g). The spin columns were placed into new 2 ml collection tubes and centrifuged for 1 min at 10,000 rpm (8,000 g). Following this step, the spin columns were placed in new 1.5 ml collection tubes and 50 µl RNase-free water was added. RNA was eluted from the spin column following centrifugation for 1 min at 10,000 rpm (8,000 g). Spin columns were discarded at this point, and the collection tubes capped and immediately transferred to ice. RNA samples were quantified using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific Inc.) and stored at -80 °C until needed for experimentation.

2.2.5 cDNA Synthesis

Total RNA (100 ng) was reverse transcribed into cDNA using the Transcriptor First Strand cDNA Kit (Roche Diagnostics Ltd., UK). The RNA was added to a thin walled PCR tube on ice and the following reagents are added to generate a 20 µl reaction: Random Hexamer primer 2 µl (60 µM); transcriptor reverse transcriptase reaction buffer (5X) 4 µl; Protector RNase Inhibitor (40 U/µl) 0.5 µl; Deoxynucleotide mix 2 µl; Transcriptor reverse transcriptase (20 U/µl) 0.5 µl. The volume was increased to 20 µl with RNAse-free as required. Cycling conditions are

shown in Table 2.1. On completion of the cDNA reaction, samples were stored at -20 °C until needed for further experimentation.

Temperature (°C)	Time (Minutes)	Description
25	10	Annealing
50	60	Elongation
85	5	Reverse Transcriptase Inactivation

Table 2.1: Cycling Conditions for generation of cDNA

2.2.6 Quantitative real-time PCR (qPCR)

Custom designed probes were generated, via Roche's RealTime ready Configurator (Roche Diagnostics, West Sussex, UK) (see Table 2.2). qPCR was performed on a Lightcycler 480 system (Roche Diagnostics Ltd., UK). Beta-actin was selected as a house-keeping gene after optimizing against a panel of potential genes. To do this, 2 μl of cDNA was added to each well of a 96-well plate, along with 5 μl of Master Mix (Roche Diagnostics Ltd., UK), 2 μl of RNase-free water and 1 μl of the relevant probe, final concentration of probe is 2x, to make a total reaction volume of 10 μl. The plate was then sealed with clear plastic film and centrifuged at 1500 rpm (226 g) for 2 min at 18 °C. The plate was read according to an optimized protocol for monocolour hydrolysis probes provided by Roche, UK (see Table 2.3).

Table 2.2: qPCR Primer Probes

Gene	Species	Assay ID
ABCC8	*R. norvegicus*	506195
	M. musculus	300187
KCNJ11	*R. norvegicus*	506200
	M. musculus	317283
KCNQ1	*R. norvegicus*	506506

	M. musculus	307946
HNF-1α	*R. norvegicus*	500240
	M. musculus	315865

Table 2.3: Cycling conditions for monocolour hydrolysis probes

Program Name	Cycles	Target °C	Analysis Mode
Pre Incubation	1	95	None
Amplification	55	95	Quantification
		60	
		72	
Cooling	1	40	None

.

2.2.7 Immunocytochemistry

BRIN-BD11 and MIN6 cells were seeded onto sterile Poly-L-lysine slides at a density of 4 x 10^4 cells/slide, and maintained in a 90 mm vented a Petri dish. Cells were allowed to attach for 4 h at 37 °C, in an atmosphere of 5 % CO_2 and 95 % air, at this point media was added to the dish for overnight incubation. Following this, cells are washed 3 x 5 minutes with PBS. Cells were incubated at room temperature for 20 min with 200 µl of ice-cold 4 % paraformaldehyde (PFA) to ensure cellular attachment. Cells were washed 3 x 5 min in PBS, and permeabalised using 0.3 % Triton-X for 15 min (for analyses of intracellular proteins). Cells were washed for a further 3 x 5 min in PBS. Cells were incubated for 10 min in blocking serum comprising 15 µl blocking serum in 1 ml PBS. Cells were then incubated overnight at 4 °C with primary antibody. Slides were not washed between the blocking stage and the addition of primary antibody, although tissue was used to drain any excess blocking serum from the slides. Primary antibody was diluted 1:100 of buffer containing 1.5 % blocking serum. Diluted antibody (200 µl) was added to each slide and covered with a piece of parafilm, cut to fit the slide, to ensure even antibody coverage and to prevent slide from drying. Primary antibody

against *SUR1*, *Kir6.2*, *KCNQ1* and *HNF-1α* (Santa Cruz Biotechnology, UK) were used.

After overnight incubation, slides were washed 3 x 5 min in PBS and 200 µl secondary antibody (Alexa Fluor® 488 goat anti-mouse IgG or Alexa Fluor® 488 goat anti-rabbit IgG – Life Technologies, UK) was added. Secondary antibody was diluted in PBS (1:400) and slides incubated for 45 min at 37 °C in a humidified chamber (place tissue soaked in PBS in corner of dish). The dish was also covered in tin foil to prevent leaching of the fluorescent signal. Slides are again washed 3 x 5 min in PBS. A total of 100 µl DAPI (stock diluted in water to give a final concentration of 125 ng/µl) was added to each slide and left at room temperature for 20 min after which a drop of Vectashield mounting medium (Vector Laboratories, Inc., Burlingame, CA, USA) was added, then covered with a coverslip. Slides were allowed to dry and sealed with nail polish. Slides were viewed using a fluorescent light source and images stored as TIFF files. Microscope used was Axio imager A.2 with an Axio MRM camera (Zeiss Group, UK).

2.2.8 Potassium Channel Inhibition

BRIN-BD11 cells were seeded into 24 well plates at a density of 15 x 10^4 cells/well and allowed to attach over night at 37 °C, in an atmosphere of 5% CO_2 and 95% air. 25 µl of antibody was then added to the applicable wells. Antibodies against SUR1, Kir6.2 and KCNQ1 (Santa Cruz, Heidelberg, Germany) were used; these are potassium channel proteins on the surface of the β-cell. After a further 24 h incubation an acute insulin response test was performed, as detailed in section 2.2.2, and samples were then stored at -80 ◦C for further analysis. By inhibiting potassium channels a simple assessment can be made of their importance to insulin secretion from the β-cell, thus validating further examination of their role.

2.2.9 Transfection with siRNA

BRIN-BD11 cells were seeded at a density of 5 x 10^4 cells/well/24 well plate, whilst MIN6 cells were seeded at a density of 8 x 10^4 cells/well/24, and allowed to attach overnight at 37 °C, in an atmosphere of 5 % CO_2 and 95 % air. Following overnight incubation, media was removed from the cells and replaced with 500 µl fresh media prior to transfection.

Custom FlexiTube siRNA (QIAGEN, Manchester, UK) was designed against *SUR1*, *KIR6.2*, *HNF-1α* and *KCNQ1* (see Table 2.4 for details). The likelihood of success of the siRNA was assessed using an online tool SpliceCenter-siRNA Check (Ryan et al., 2008). Following an extensive optimization process, which is summarized in Table 2.5, 100 nM siRNA was added to an eppendorf tube containing 100 µl of serum-free culture medium, along with 2.5 µl Lipofectamine Transfection Reagent (Invitrogen, UK). The mixes were incubated for 15 min at room temperature to allow transfection complexes to form. These complexes were then added drop-wise onto the cells (to give a final volume of 600 µl per well), which were then incubated under normal growth conditions for 48 h (BRIN-BD11) or 72 h (MIN6). Mock transfections and scrambled siRNA were used as controls in all experiments. Gene silencing was assessed by qPCR as described above.

siRNA Name	ID
Mm_ABCC8_1	SI02759911
Mm_KCJ11_4	SI00271187
Mm_KCNQ1_1	SI00175812
Mm_HNF1a_1	SI04746560
Mm_Tcfl_2	SI014444058
Mm_Tcfl_4	SI01444072
Rn_ACC8_5	SI03091977
Rn_KCNJ11_4	SI00194285
Rn_KCNQ1_1	SI03033492
Control (non-sil.) siRNA	1022076

Table 2.4: Custom FlexiTube siRNA assay IDs (Qiagen, Manchester, UK)

Cell Seeding Density	Transfection Time (Hours)	siRNA Concentration (nM)	Transfection Reagent (µl)	% Knockdown
100,000	24	10	3	20
120,000	24	50	3	35
120,000	48	50	3	40
120,000	72	100	3	40
50,000	72	100	2.5	80-90
80,000	72	100	3.5	60-90

Table 2.5: siRNA optimization

2.2.10 Determination of cell viability by colorimetric 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay

An MTT assay was used to assess cell metabolic activity. The tetrazolium dye MTT is added to cells and taken up by the mitochondria in viable cells, where it is reduced to purple formazan. Following the addition of the solubilisation solution DMSO, the purple formazan is dissolved into a coloured solution. The absorbance of this solution can then be read and quantified (Stockert et al., 2012). BRIN-BD11 cells were seeded and transfections performed as per section 2.2.8. The calorimetric MTT assay was then used to determine cell metabolic activity following siRNA

transfection at 24, 48 and 72 h. The media was removed from the cells, and replaced with 400 µl of media containing 40 µl of 12 mM MTT stock solution. The cells were further incubated for 2 h at 37 °C. MTT-containing media was then removed from each well, and replaced with 400 µl of DMSO, and the plate was returned to the incubator for a further 45 minutes. Absorbance was then read at a wavelength of 570 nm with correction at 630 nm using an Epoch Microplate Spectrophotometer (BioTek, UK).

2.2.11 Cellular protein extraction

Cells were harvested and pelleted as described in Section 2.2.1. The supernatant was then removed and 100 µl of ice-cold protein extraction buffer (RIPA Buffer (Sigma Aldrich, UK)) supplemented with Pierce™ Protease and Phosphatase Inhibitor mini tablets (1 tablet per 10 ml RIPA buffer) was used to resuspend the cell pellet, followed by incubation on ice for 20 min with vortexing of the lysate every 5 min. The lysate was then centrifuged at 12,700 rpm (18,213 g) for 15 min at 4 °C. Supernatant containing the cellular protein was then transferred to a fresh tube and stored at -80 °C for future use.

2.2.12 Protein quantification

Total protein was determined using the Thermo Scientific Pierce BCA Protein Assay Kit (Thermo Fisher Scientific (UK). This assay uses the reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline medium with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{1+}) by bicinchoninic acid (BCA). The intensity of the colour is directly proportional to the concentration of the protein within the sample.

Protein samples were thawed on ice and 10 µl of each was added into a clean, dry, 96-well microplate. A total of 200 µl of working reagent was added to all samples and standards. Working reagent was prepared by combining 50 parts of BCA reagent A with 1 part BCA reagent B (50:1, Reagent A:B). The plate was then gently agitated for 30 seconds, after which it was covered and placed in an incubator at 37 °C for 30 min. After the plate had cooled to room temperature, the absorbance was measure at 562 nm on a plate reader. A typical standard curve is shown below in Figure 2.4

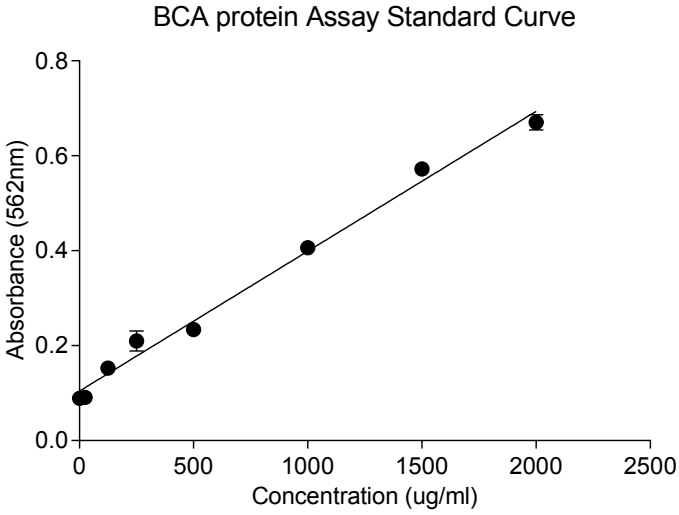


Figure 2.4 – Typical BCA assay standard curve

A standard curve was created ranging from 0 - 2,000 µg/ml using bovine serum albumin (BSA) standards provided.

2.2.13 Patient Recruitment to the DIASTRAT cohort

The Stratified Medicine optimizing treatment for Diabetes (DIASTRAT) study is a pilot observational study, investigating the incidence of variation of candidate biomarkers within a local type 2 diabetes population. A total of 500 people were asked to participate in the study and were recruited from the Western Health and Social Care Trust. Criteria for inclusion on the study were as follows: aged between 18 and 80 years; diagnosed with type 2 diabetes. Those outside the age limits, or with other forms of diabetes (type 1; secondary diabetes) were excluded

from participation. Those approached to participate had been previously diagnosed with T2DM and were identified by the consultant doctors at Altnagelvin hospital as being eligible to participate and then were contacted by those working on DIASTRAT to be asked to participate, or were brought to the DIASTRAT team while attending their diabetes clinic at Altnagelvin hospital having seen their consultant doctor that day. All participants were recruited from secondary care. The majority of participants were recruited from the same area, with a large proportion residing within one postcode area of Derry City. All participants provided informed written consent and the study was approved by the Office for Research Ethics Committees Northern Ireland (ORECNI) on 12/11/2014. (ORECNI Ref: 151917; NHS/HSC Trust Ref: WT 14/25 151917; HSC Rec Ref: 14/NI/1123). The Invitation letter, participant information sheet and study protocol are provided in Appendix 1. The recruitment process relied on input from others, applying for ethical approval for the study, identifying or contacting participants, collecting and processing samples, and finally to collating participant information stored in their medical records. As such recruitment was performed by this author (Declan McGuigan), Andrew English (PhD student, Ulster University), Dr Catriona Kelly (Ulster University) Dr Paula McClean (Ulster University), Dr Geraldine Horrigan (C-TRIC), Dr Maurice O'Kane (WHSCT), Dr Neil Black (WHSCT), Dr Athinya Thiraviaraj (WHSCT).

2.2.14 Processing blood samples

Consented participants each provided 2 x 9 ml tubes (containing EDTA) and 1 x 8 ml tube serum separating tube. One 9 ml EDTA tube was frozen at – 80 °C, while the second EDTA tube and the serum separating tube were processed immediately. The blood samples were centrifuged for 15 min at 3000 rpm at 4 °C. Serum, from the serum separating tubes, was removed and aliquoted into individually labeled 1.5ml eppendorf tubes and stored at -80 °C. Centrifugation of the tubes containing EDTA,

produced 3 layers: an upper plasma layer, a middle buffy coat layer (containing leukocytes and white platelets) and a lower red blood cell layer. The plasma layer was taken and aliquoted into individually labeled 1.5ml eppendorf tubes and stored at -80 ◦C. The middle buffy coat layer was removed into a separate 1.5 ml eppendorf tube, to which 1 ml of PBS was added, and the contents then split evenly between two 1.5 ml eppendorf tubes. The buffy coat in each tube was washed twice with PBS, and the remaining red blood cells removed. RNALater (1 ml; Ambion) was added to one buffy coat tube, and 1 ml of M-PER™ Mammalian Protein Extraction Reagent (ThermoFisher Scientific) was added to the other buffy coat tube. Samples were then stored at -80 ◦C until needed for further analysis.

2.2.15 DNA extraction from buccal swabs

Buccal swabs were collected from consenting participants using a Dacron swab to brush the inside of the cheek. This was then collected in a 15ml Falcon tube and processed. To extract DNA, the Qiagen QIAmp DNA Mini kit (Qiagen (Manchester, UK) was used. 600 µl of PBS was added to the tube containing the swab, along with 20 µl Qiagen Protease stock solution and 400 µl Buffer AL. The sample was then immediately mixed by vortexing for 15 s. The sample was then incubated at 56 ◦C for 10 min. Ethanol (400 µl) was then added, and the sample mixed again by vortexing. Following this, 700 µl of the sample was carefully removed to a QIAmp mini spin column and centrifuged at 8000 rpm (6000 x g) for 1 min. The column was then placed in a fresh collection tube, and 400 µl of ethanol was again added to the swab and mixed. A total of 700 µl of the remaining sample from the Falcon tube was added to the spin column. Buffer AW1 (500 µl) was then added to the spin column and centrifuged at 8000 rpm (6000 x g) for 1 min. The column was placed in a fresh collected tube, and 500 µl of Buffer AW2 added. Subsequently the column was centrifuged at full speed (14,000

rpm or 20,000 x g) for 3 min. The spin column was finally placed in a clean 1.5 ml microcentrifuge tube, and 150 µl of Buffer AE added. This was then kept at room temperature for 1 min before a last centrifuge at 8000 rpm (6000 x g) for 1 min. Samples were quantified using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific Inc.)

2.2.16 DNA extraction from whole blood

The extraction of DNA from whole blood was performed using the Qiagen Gentra Puregene Blood Kit. A total of 300 µl of either fresh or frozen whole blood can be used. Initially, 900 µl of RBC lysis solution was added to a 1.5 ml microcentrifuge tube, followed by 300 µl of whole blood. This was then mixed by inverting ten times, and incubated for 1 min at room temperature, inverting at least once during incubation. The sample was then centrifuged for 20 s at 13,000 – 16,000 x g to pellet the white blood cells. The supernatant was then carefully discarded through pipetting or pouring, leaving approximately 10 µl in the tube, and the white blood cell pellet. The tube was then vortexed vigorously to resuspend the pellet in the residual liquid to aid cell lysis, which was achieved through the addition of 300 µl of cell lysis solution. The liquid was pipetted up and down to lyse the cells, followed by vigorous vortexing for 10 s. To give an RNA-free DNA sample, 1.5 µl of RNase A solution was added, and the sample incubated for 15 min at 37 °C. The sample was then quickly cooled on ice for 1 min. A total of 100 µl of protein precipitation solution was added, followed by 220 s of vortexing at high speed. The sample was centrifuged for 1 min at 13,000 – 16,000 x g, with the precipitated protein forming a tight, dark brown pellet. Isopropanol (300 µl) was added to a clean 1.5ml microcentrifuge tube, to which the supernatant from the previous step was added, with caution taken to not dislodge the protein pellet.

Next the sample was then mixed by inverting 50 times, until the DNA was visible as threads or a clump. This was followed by another centrifugation step for 1 min at 13,000 – 16,000 x g, producing a small white DNA pellet. The supernatant was then carefully discarded and the tube carefully drained onto clean absorbent paper, making sure the pellet remains in the tube. At this point, 300 µl of 70% ethanol was added and the sample inverted several times to wash the pellet, followed by a further centrifugation step for 1 min at 13,000 – 16,000 x g. Again, the supernatant was discarded, the tube drained onto clean absorbent paper, with care taken not to dislodge the pellet, and the pellet left to air dry for 5 min. Then 100 µl of DNA hydration solution was added, and vortexed for 5 s at a medium speed to mix, and incubated at 65 °C for 5 min to dissolve the DNA. Finally, the sample was incubated overnight at room temperature, with gentle shaking, after which the sample was briefly centrifuged and transferred to a storage tube before being quantified, samples were quantified using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific Inc.)

2.2.17 Human Insulin ELISA for analysis of plasma samples

The Mercodia Insulin ELISA (Mercodia, Uppsala, Sweden) was used for the quantitative determination of insulin from human plasma samples. The Mercodia Insulin ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. To begin, 25 µl of each calibrator, sample and control was added to the appropriate well. Following the addition of samples, 100 µl of enzyme conjugate was added; the plate was covered in foil and placed on a plate shaker at 900 rpm for 1 hour at room temperature. Each well was then washed 6 times with 350 µl of wash buffer. Working strength wash buffer was prepared by diluting wash buffer concentrate that had been supplied with 20 parts distilled water.

Substrate TMB (200 µl) was added to each well, and the plate further incubated at room temperature for 15 min. Finally, 50 µl of stop solution was added and absorbance was read immediately at 450 nm using an Epoch Microplate Spectrophotometer (BioTek, UK). A typical standard curve is illustrated in Figure 2.5.

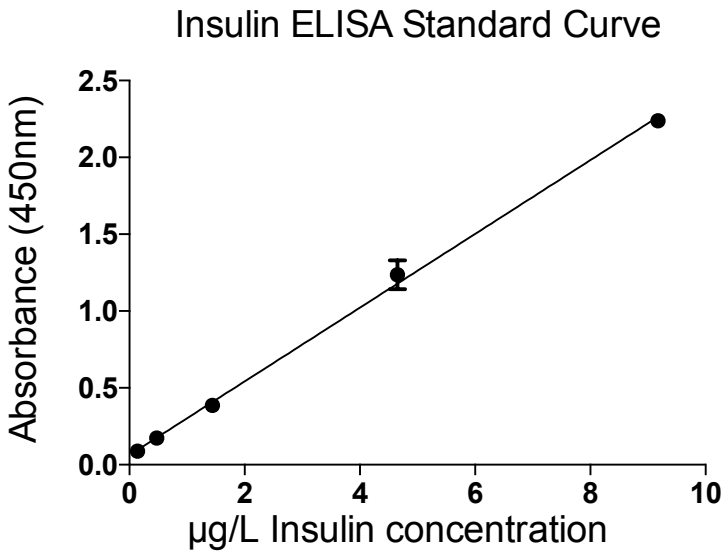


Figure 2.5: Typical Human Insulin ELISA standard curve

An insulin standard curve ranging from 0 – 9.17 µg/L was created with the standards provided.

2.2.18 Human C-peptide ELISA for analysis of plasma samples

The ALPCO (Newmarket, UK) C-peptide ELISA was used for the quantitative determination of C-peptide in human plasma samples. The 96-well microplate is coated with a monoclonal antibody specific for C-peptide. To start, 25 µl of each standard, control and sample was pipetted into their respective wells. Assay buffer (50 µl) was then added to each well. The microplate was then covered with a plate sealer and incubated at room temperature for 1 hour, with shaking at 700 – 900 rpm. After this, the contents of the plate were decanted and the plate washed 6 times with 350 µl of working strength wash buffer in each well. Following this, 100 µl of working strength conjugate was added to each

well, the plate was then covered again, and placed on a shaker at 700 – 900 rpm for 1 hour at room temperature. The plate was again washed 6 times with 350 µl of wash buffer. 100 µl of TMB substrate was then added to each well, and the plate was incubated for 15 min at room temperature on a shaker at 700 – 900 rpm. Finally, 100 µl of stop solution was added to each well, and absorbance was read immediately at 450 nm using an Epoch Microplate Spectrophotometer (BioTek, UK). A typical standard curve is illustrated in Figure 2.6.

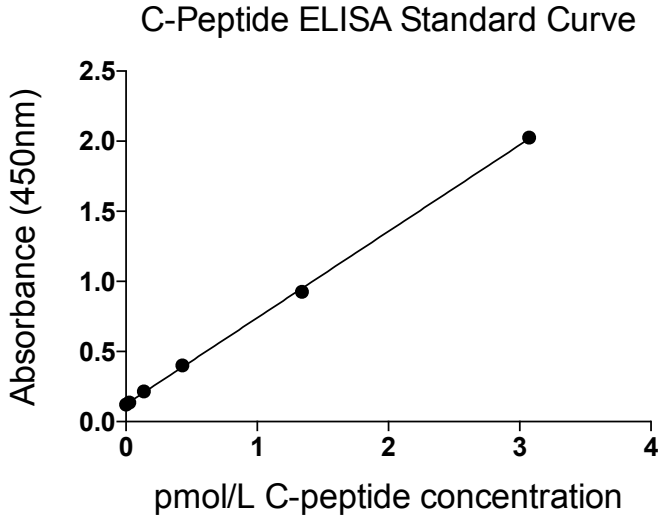


Figure 2.6: Typical Human C-peptide ELISA standard curve

A C-peptide standard curve ranging from 0 – 3000 pmol/L was created with the standards provided.

2.2.19 Database construction

Following recruitment of participants, clinical, anthropometric and biochemical information was collected for each patient. This included a lipid profile, HbA1c history, and all current prescribing information. This information was collected from two sources within the Western Health and Social Care Trust (WHSCT). The DIAMOND database and the Northern Ireland Electronic Care Record (NIECR) database were both consulted to give a full history. DIAMOND is a database used in the WHSCT to collect all relevant clinical information specifically from

diabetes patients' electronic records. Whereas NIECR is the electronic care record system for all patients in NI, and houses all clinical information – details of hospital visits, test results etc – in one place for authorized personnel to access. Where possible data was entered in binary form, and where it was needed internationally recognised standard units were used. Figure 2.7 below shows select parts of the database, to provide a better understanding of the volume of data that was being collected from patient records.

Last Episode Date (seconary care)	Last HbA1c (IFCC) Lab Result	Last Weight (Kg)	Last BP Systolic (Sitting) mmHg	Last BP Diastolic (Sitting) mmHg	Last HDL Lab Result (mmol/l)	Last LDL Lab Result (mmol/l)	Last Cholesterol Lab Result (mmol/l)	Last Creatinine (umol/l)	Last BMI	Last Height (cm)
25/02/2016	77.0000	93.00	136.00	78.00	1.0000	1.5000	3.0000	116.0000	31.44	172
16/12/2015	57.0000	102.00	134.00	68.00	1.3000	1.4000	3.0000	163.0000	39.35	161
16/12/2015	57.0000	102.00	134.00	68.00	1.3000	1.4000	3.0000	163.0000	39.35	161
05/08/16	79.00	110.00			0.80	2.00	3.80	77.00	34.35	180.00
17/01/17	80.00	63.50	122.00	68.00	1.11	0.74	3.20	74.00		
23/02/2016	62.0000	75.00	124.00	50.00	1.0500	1.7300	3.6000	128.0000	26.26	169
25/02/2016	76.0000	97.00	130.00	74.00	0.7000	0.9000	2.9000	104.0000	34.78	167
26/01/2016	65.0000	99.00	136.00	66.00	0.9200	1.7300	3.7000	146.0000	31.96	176
27/08/2015	47.0000	91.40	122.00	72.00	1.2200	1.4400	3.2000	106.0000	30.90	172
15/02/2016	79.0000	83.00	130.00	72.00	1.0000	1.3000	2.7000	71.0000	28.72	170
07/12/2015	58.0000	70.40	137.00	78.00	1.1000	2.1700	4.5000	175.0000	28.20	158
01/06/2016	66.0000	79.30	156.00	80.00	1.6400	3.8300	7.5000	89.0000	30.98	160
20/07/15										
11/11/2015	55.0000	100.30	144.00	86.00	0.8400	2.8900	4.6000	165.0000	38.69	161
19/02/2016	65.0000	94.00	128.00	70.00	1.4000	1.5000	3.2000	137.0000	31.05	174
25/07/2016	80.00	71.80	134.00	78.00	1.5000	1.5000	3.5000	98.0000	27.02	163
14/12/16	94.00	89.30	120.00	80.00	1.47	2.49	4.40		30.90	170.00
13/01/2016	68.0000	93.00	133.00	70.00	1.0000	0.4000	2.4000	131.0000	37.25	158
04/12/15	81.00	137.00	144.00	64.00	1.20	1.40	3.20	94.00	47.40	170.00
20/01/2016	61.0000	84.20	124.00	76.00	0.6000	0.9000	1.9000	127.0000	25.14	183
17/09/15	73.00	85.70							31.48	165.00
25/02/2016	62.0000	83.00	137.00	76.00	1.0000	1.1000	2.7000	154.0000	27.41	174
20/01/2016	65.0000	65.30	138.00	74.00	1.8000	1.2400	3.7000	63.0000	22.33	171
10/06/15	63.00	103.00	134.00	74.00	1.3000	1.1000	2.9000	78.0000	34.09	
06/12/16	59.00	121.56			1.20	1.50	3.20	78.00		
16/12/16	70.00	87.20			0.60	1.10	2.60	76.00		
15/10/2015	56.0000	91.80	112.00	60.00	1.0900	1.6700	3.7000	165.0000	29.64	176
08/11/16	66.00	78.00								
11/02/2016	65.0000	82.90	144.00	66.00	1.1000	2.3000	5.3000	150.0000	30.08	166
19/06/16	58.00	74.00	130.00	70.00	1.21	1.96	3.70	96.00	26.22	168.00
20/09/16	75.00	91.00	142.00	82.00	1.00	1.30	3.40	111.00	32.63	167.00
10/02/17	55.00	97.20	150.00	80.00	1.03	1.63	3.20	60.00		
05/02/2016	47.0000	79.20	126.00	74.00	2.0800	0.8000	3.5000	99.0000	29.45	164
28/07/2016	59.0000	78.20	125.00	76.00	1.8200	1.1700	3.2000	77.0000	24.14	180
01/09/16	56.00	71.00			1.30	2.40	4.90	62.00		
10/03/2016	67.0000	104.00	128.00	76.00	0.7000	1.7800	4.1000	109.0000	32.82	178
14/01/15	57.00	102.00	131.00	76.00	1.4000	1.5000	3.4000	96.0000	33.31	
14/10/16	60.00	61.70	140.00	80.00	1.30	2.20	4.30	93.00	22.12	167.00
19/10/16	74.00	68.00	139.00	81.00	1.10	1.90	3.60	46.00	25.59	163.00

Figure 2.7: Database Construction

Above are screenshots of the DIASTRAT patient information database, showing just some of the anthropometric and clinical data that was collected for participants in this study.

Teatment1	Dose	Started	Time on (days)	Treatment2	Started
NovoRapid	22units	06/09/12	1414	Levemir	06/09/12
Toujeo Solostar	100nits	08/12/15	227	Humalog KwikPen	17/07/14
Metformin	2000	18/07/01	5480	Glimepiride	13/03/12
Metformin	500	06/02/02	5286	Gliclazide MR	21/01/09
Glucophage SR	1000	05/10/06	3155	Pioglitazone	02/11/06
NovoRapid flexpen	Pen	27/07/12	1455	Lantus Solostar	13/04/15
Novomix 30 Flexpen	20units	13/06/06	3689	Metformin	18/04/02
Lantus Solostar	8units	26/02/15	509	Metformin SR	14/03/11
Competact	1	22/01/14	489	Gliclazide	11/03/15
Glucophage SR	1000	04/06/14	356	Gliclazide	04/06/14
Lantus Solostar	Pen	25/05/15	421	Gliclazide	26/06/14
NovoRapid flexpen	18	21/02/13	1251	Metformin SR	21/02/13
Liraglutide	0.6	29/10/10	1670	Metformin	01/05/12
Novomix 30 Flexpen	18/50	29/09/14	659	Metformin SR	24/03/15
Sitagliptin	100	25/09/14	665	Metformin	25/09/14
Metformin	500	02/11/04	4322	Sitagliptin	05/07/16
Lantus Solostar	10	04/03/15	512	Metformin	23/02/10
Gliclazide	160	01/12/14	178	Metformin	01/12/14
Glucophage SR	2000	20/03/08	2625	Gliclazide	20/03/08
Metformin	1000	13/05/13	745	Liraglutide	14/05/13
metformin	1000	24/06/06	3265	Gliclazide	07/03/12
Humulin M3 cartridge	16	29/01/02	5288	Metformin	11/05/06
Metformin	500	25/06/09	2581	Dulaglutide	14/03/16
Glucophage SR	2000	10/07/07	2918	Novomix 30 flex	08/09/04
Lantus Solostar	16	02/12/13	969	Gliclazide MR	30/09/13
Novomix 30 Flexpen	32	16/07/08	2546	Metformin	16/07/08
Metformin	1000	29/09/11	1757	Liraglutide	23/11/11
Liraglutide	1.2	11/03/10	2324	Metformin	01/03/05
Novomix 30 Flexpen	30	05/11/12	977	Metformin	23/01/02
Lantus Solostar	40	04/11/13	631	Humalog KwikPen	04/11/13
Novorapid penfil	14	20/11/03	4627	Lantus Solostar	02/05/12
novorapid flexpen	8	14/02/13	894	Levemir flexpen	16/07/13
Novomix 30 Flexpen	56	09/06/05	3701	Glucophage SR	28/07/08
Novomix 30 Flexpen	70	18/06/12	1135	Sitagliptin	20/08/15
Liraglutide	1.2	24/09/12	1396	Dapagliflozin	25/02/15
Metformin	1g	30/07/13	728	Saxagliptin	18/04/14
Novamix 30 FLEX	38	23/07/07	3293		
Humalog mix 50 kwikpen	46	01/11/10	2090	Humalog mix 25 kwikpen	06/02/12
Metformin	1000	10/07/03	4401		
Novomix 30 Flexpen	16 / 38	15/01/07	3116	metformin	15/01/07
Liraglutide	1.2mg	02/05/13	1174	metformin	02/05/13
Novomix 30 flexpen	15	04/05/16	78	Glucophage SR	2000
Liraglutide	1.2	13/11/09	2440	Metformin SR	2000
Novorapid pen	20-30	07/10/04	4305	Lantus cartridge	50

Figure 2.8: Database Construction

Above are screenshots of the DIASTRAT patient information database, showing just some of the anthropometric and clinical data that was collected for participants in this study.

2.2.20 OLINK Proximity Extension Assay

Proximity extension assay (PEA) work was outsourced to Olink Bioscience, Uppsala, Sweden. They use high-multiplex immunoassays for targeted human protein biomarker discovery. The technology involved can

measure 92 proteins across 96 samples simultaneously from 1 µl of plasma. A pair of oligonucleotide-labeled antibodies, Proseek probes, are allowed to pair-wise bind to the target protein present in the sample in a homogeneous assay. When the two Proseek probes are in close proximity, a new PCR target sequence is formed by a proximity-dependent DNA polymerization event. The resulting sequence is subsequently detected and quantified using standard real-time PCR. Each of the 96 oligonucleotide antibody-pairs contains unique DNA sequences allowing hybridization only to each other. Subsequent proximity extension creates 96 unique DNA reporter sequences that are amplified by real-time PCR. Cross-reactive events are not detected with Olink's panels since only matched DNA reporter pairs are amplified with real-time PCR.

As described above in Section 2.2.16, plasma was separated from blood taken from participants in this study, 50 µl of plasma from each participant was then loaded into one well of a 96 well plate. These plates were then shipped to Olink, Uppsala, Sweden for analysis.

2.2.21 SNP genotyping using the Affymetrix UK Biobank array

DNA extracted from whole blood was quantified using the Qubit Fluorometric Quantitation (ThermoFisher Scientific Inc.). The Qubit allows for quantitation of dsDNA using fluorescence-based quantitation assays. As quality control, a subset of samples were also run on a 1% agarose gel to check for any degradation prior to sending for sequencing (Figure 2.8). These sample were also measured using the NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific Inc.) to confirm the OD260/OD280 ratio was between 1.8 and 2.0 and the OD260/OD230 was greater than 1.5.

Samples were required to have an input mass of 100 ng, gDNA concentration of 5 ng/µl, with a final volume of 20 µl. DNA samples were diluted using reduced EDTA TE buffer (10mM Tris-HCL pH 8.0, 0.1 mM EDTA), which was supplied by Affymetrix. Samples were aliquoted into ABgene 96 Square Well Storage (Thermo Fisher Scientific Inc.) and sealed with caps mats (Thermo Fisher Scientific Inc.). Samples were shipped on dry ice to Cambridge Genomics Services (CGS) for analysis on the Affymetrix UK Biobank array. CGS is a core facility within the Department of Pathology at Cambridge University that offer genomics technologies and bioinformatics support.

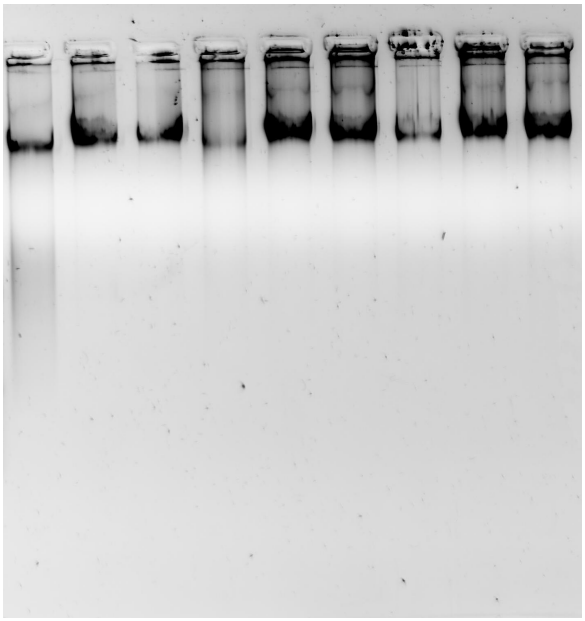


Figure 2.9: DNA Gel

A subset of DNA samples were run on a 1% agarose gel to assess DNA quality prior to being sent for sequencing.

2.2.22 Statistical Analyses

Data are presented as mean ± standard error of the mean (SEM) or standard deviation (SD) for a given number of observations (N) as indicated in the Figures. Comparison of gene and protein expression between groups was compared by two-tailed unpaired Student *t* test (for data standardized against a control) or one-way ANOVA with

posthoc test (for non-standardised data) using either Graphpad, (PRISM Software, USA) or SPSS Statistics (IBM software). Correlations were interrogated using Pearson's or Spearman's coefficient (SPSS) as appropriate. Significance was accepted at $P<0.05$.

Analysis of Olink data was completed in Matlab with bespoke scripts written by Dr Steven Watterson (Stratified Medicine, Ulster University). Proteins falling below the level of assay detection were excluded from the analysis. Expression of the remaining proteins between control and DIASTRAT groups were compared using an unpaired two-sample *t* test with significance accepted at $P<0.05$. The proteins that passed this test were ranked, with the smallest overlap in the distributions between the patient groups at the top of the list and the proteins with the largest overlap at the bottom. The top 75 proteins were selected and the linear combination sought to enable calculation of a pseudo-protein with the smallest possible overlap in the distributions between the two participant groups.

Analyses of UK Biobank array genotyping data was performed using Axiom's Genotype Console software. Samples failing quality control (SNP call rate <97.2% and QC<0.82) were excluded from further analysis. Variant calling was performed following creation of SNP lists against each gene of interest. Using XLSTAT, comparisons between genotypes were assessed using a two-sample *t* test for two proportions following conversation of percentages positive for each genotype to proportions (where 100% = 1) and 95% confidence interval (CI). Significance was accepted at $P<0.05$. Clustering of genotypes of interest was performed using Genotype Console software (Thermo Fisher Scientific, UK).

Chapter 3:

Genetic drivers of sulphonylurea response in β-cell lines

3.1 Introduction

Sulphonylureas (SU) are a commonly used treatment in T2DM. They work by acting directly on the pancreatic β-cell to stimulate insulin secretion (Proks et al., 2002). They target the ATP-sensitive potassium (K_{ATP}) channel, which is a hetero-octameric complex comprising two types of protein subunit – an inwardly rectifying potassium subunit (Kir6.2), and sulphonylurea receptor 1 (SUR1) (Ashcroft and Gribble, 1999, Proks et al., 2002). Subsequent inhibition of this channel triggers membrane depolarisation of the pancreatic β-cell, prompting the opening of voltage-gated Ca^{2+} channels, which in turn allows entry of Ca^{2+} in to the pancreatic β-cell, and stimulates the exocytosis of insulin from secretory granules (Proks et al., 2002). The most common adverse event observed with SU is hypoglycaemia. For some individuals this can be severe and persistent due to the duration of action of the drug. This is particularly troublesome with first generation sulphonylureas like tolbutamide and chlorpropamide (Aquilante, 2010).

With SU treatment, interindividiual variation in response is a concern. It is estimated that 10-20% of patients will suffer from primary SU failure; that is, they will have less than a 20 mg/dl reduction in fasting plasma glucose (DeFronzo, 1999, Aquilante, 2010). Roughly 50-60% of patients will ultimately fail to reach the required glycaemic control following a primary decrease in fasting plasma glucose of greater than 30 mg/dl (DeFronzo, 1999, Aquilante, 2010). Secondary SU failure is also an issue in around 5-7% of patients who have a good response at the start (DeFronzo, 1999, Aquilante, 2010). This varying response has also been replicated in large-scale randomized trials; in particular the A Diabetes Outcome Progression Trial (ADOPT) revealed a sulphonylurea monotherapy failure of 34% contrasted with 21% for metformin (Kahn et al., 2006, Aquilante, 2010).

Along with this variation in the response to treatment, variation is also seen in occurrence of adverse events. Approximately 31% of patients suffer mild hypoglycaemia in the first year of glibenclamide treatment, with the frequency of severe hypoglycaemia estimated at approximately 1% per year (UKPDS Group, 1998). There are clinical factors involved in the failure of SU treatment including deteriorating β-cell function, long-standing diabetes, elevated baseline glucose levels and an elevated degree of insulin resistance (Aquilante, 2010). There are also some factors that affect severity of SU induced hypoglycaemia, including the duration of sulphonylurea action, mild baseline hyperglycemia, irregular eating patterns, excessive alcohol intake and age (Aquilante, 2010).

Clinical factors are not the sole regulators of variation to response. There is evidence suggesting certain single nucleotide polymorphisms (SNPs) are involved (Aquilante, 2010). However, there is little consensus in the literature as to what SNPs are at play. This is highlighted in the work of Song et al. (2017), who identified SNPs in *ABCC8*, *KCNJ11*, which have evidence showing, or not, an association with T2DM (Meirhaeghe et al., 2001, Zychma et al., 2002, Ragia et al., 2012, Li et al., 2014). Similar effects were observed for *KCNJ11* alone, with Haghvirdizadeh and colleagues citing multiple SNPs involved in SU response, which was again supported and contrdicted by other studies (Haghvirdizadeh et al., 2015). The lack of consensus and among these groups perhaps highlights difference of effect of SNPs among different populations.

The aim of this study therefore was to assess the effect of ABCC8, *KCNJ11*, *KCNQ1*, *HNF1α* and *TCF7L2* deficiency on SU (glibenclamide and tolbutamide) response from single β-cells using two β-cell lines: MIN6 and BRIN-BD11. The hypothesis therefore being tested was that deficiency, or knockdown in expression, would cause a reduction in insulin output from the two cell lines.

3.2 Materials and Methods

3.2.1 Chemicals

Reagents of analytical grade and deionized water were used. All other chemicals used are listed in Chapter 2, Section 2.1

3.2.2 Culture of β-cell lines

Two pancreatic β-cell lines, MIN6 and BRIN-BD11, were grown and maintained in DMEM and RPMI tissue culture medium respectively, as described in Chapter 2, Section 2.2.1.

3.2.3 RNA extraction and Quantification

Total RNA was extracted from monolayers and quantified as described in Chapter 2, Section 2.2.4.

3.2.4 cDNA synthesis

Total RNA (100 ng) was reverse transcribed into cDNA using the Transcriptor First Strand cDNA Kit (Roche Diagnostics Ltd., UK) as described in Chapter 2, Section 2.2.5.

3.2.5 Quantitative real-time PCR (qPCR)

Quantitative real-time PCR (qPCR) was performed as described in Chapter 2, Section 2.2.6.

3.2.6 Immunocytochemistry

Immunocytochemistry was performed using primary antibodies for SUR1, Kir6.2, KCNQ1 and HNF1α according to the protocol described in Chapter 2, Section 2.2.7.

3.2.7 Assessment of acute insulin release from Pancreatic β-cells

Release of insulin from MIN6 and BRIN-BD11 cells, directly, or following transfection with siRNA, was assessed using the method described in Chapter 2, Section 2.2.3. Following 40 min preincubation with 1.1 mM

glucose, cells were acutely exposed to 16.7 mM glucose with 200 μM SU (Tolbutamide or Glibenclamide) for 20 min.

3.2.8 Protein Extraction and Quantification

Total protein was extracted as described in Chapter 2, Section 2.2.12. Protein was measured using the quantitative BCA assay, described in Chapter 2, Section 2.2.12. For the purposes of these experiments, protein concentrations were determined following acute tests and insulin secretory data standardized for protein content.

3.2.9 Potassium Channel Inhibition

BRIN-BD11 potassium channel inhibition was performed following addition of 25 μl antibodies again SUR1 (encoded by ABCC8), Kir6.2 (Encoded by KCNJ11) and KCNQ1 as described in Chapter 2, Section 2.2.8.

3.2.10 siRNA Transfection

Pancreatic β-cell lines MIN6 and BRIN-BD11 were transfected with siRNA against ABCC8, KCNJ11, KCNQ1 and HNF1α as described in Chapter 2, Section 2.2.9.

3.2.11 Statistical Analyses

Results are presented as mean ± SEM for a given number of observations (n) as indicated in the Figures. Groups of data were compared using unpaired Student's *t*-tests or One-way ANOVA with posthoc tests. Significance was accepted if $P<0.05$.

3.3 Results

3.3.1 BRIN-BD11 and MIN6 pancreatic β-cell lines secrete insulin following exposure to sulphonylureas

BRIN-BD11 (Figure 3.1A) and MIN6 (Figure 3.1B) cells were exposed to 16.7 mM glucose and tolbutamide or glibenclamide (200 μM) for 20 min. BRIN-BD11 cells displayed a 319.16 ± 12.3% increase (P<0.005) and a 568.99 ± 10.3% increase (P<0.0001) in insulin secretion in response to tolbutamide and glibenclamide respectively (Figure 3.1A). Similar data was obtained from MIN6 cells where insulin secretion was significantly enhanced in response to both sulphonylureas (Figure 3.1B)

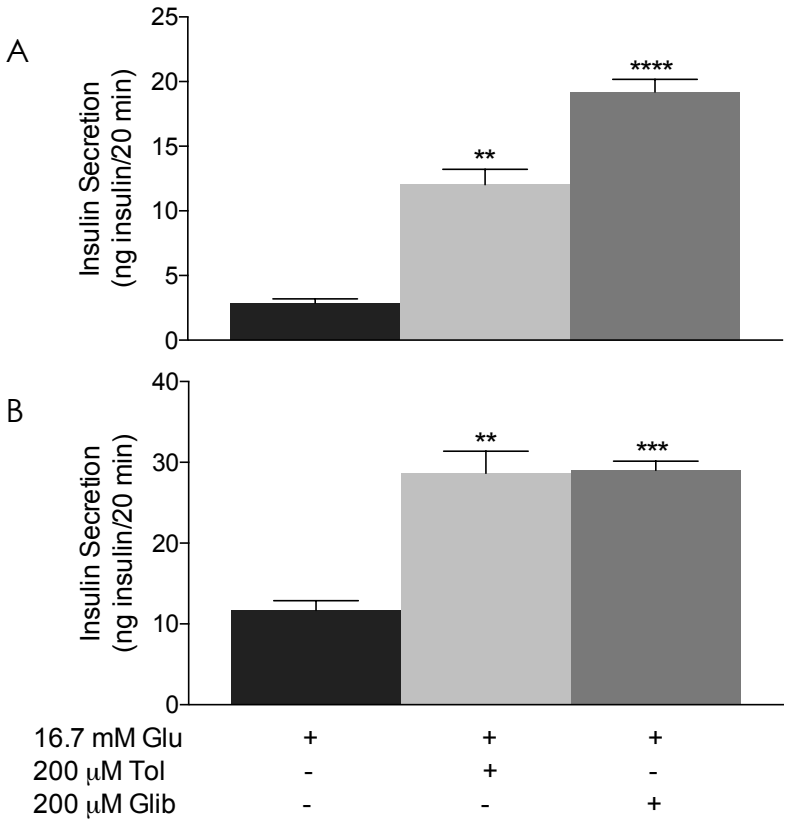


Figure 3.1: Sulphonylurea-potentiated insulin secretion in β-cell lines

BRIN-BD11 (A) and MIN6 (B) cells were exposed to 16.7 mM glucose and 200 μM tolbutamide or glibenclamide for 20 min and insulin secretion assessed by ELISA. Data are presented as mean ± SEM (n = 3). $^{**}P$<0.005, $^{***}P$<0.001 $^{****}P$<0.0001 compared with control (16.7 mM Glucose).

3.3.2 BRIN-BD11 and MIN6 pancreatic β-cell lines express candidate biomarkers at both the mRNA and protein level

Candidate genes reported to enhance T2DM risk and to be involved in sulphonylurea response were identified after a thorough review of the

literature and phenotypes validated using the Type 2 Knowledge Portal. Evidence in support of each gene is summarised in Table 3.1 Expression of each of the candidate genes was assessed using qPCR (Roche Lightcycler 480) with β-actin serving as a house-keeping reference gene. ABCC8, KCNJ11, HNF-1α and KCNQ1 were expressed in BRIN-BD11 and MIN6 cells (Figure 3.2). Expression of each candidate was confirmed at the protein level as shown in Figure 3.3.

Despite reports showing its important role in insulin production, and expression in the MIN6 cell line (da Silva Xavier et al., 2009, Zhou et al., 2014), TCF7L2 expression was not observed in either cell line in this study. Several primer-specific probes against both rat and mouse TCF7L2 sequences were employed. However, cp values were not observed in any instance. Given this, investigation of the role of TCF7L2 was not carried forward for further analysis in this cell line study.

Gene	T2DM association	SU response association	T2 Diabetes portal phenotype association
ABCC8	(Hani et al., 1997, Hansen et al., 1998, Hart et al., 1999, Hart et al., 2000, Rissanen et al., 2000, Reis et al., 2002, Florez et al., 2007, Hamming et al., 2009, Fatehi et al., 2012, Sokolova et al., 2015)	(Aquilante, 2010, Song et al., 2017, Feng et al., 2008, Pollastro et al., 2015)	T2DM; Height; BMI; Subcutaneous adipose tissue volume; Microalbuminaria; Proinsulin levels
KCNJ11	(Eftychi et al., 2004, van Dam et al., 2005, Florez et al., 2007, Aquilante, 2010, Gong et al., 2012, Li, 2013, Qin et al., 2013, Qiu et al., 2014, Haghvirdizadeh et al., 2015, Rastegari et al., 2015)	(Sesti et al., 2006, Holstein et al., 2009, Aquilante, 2010, El-Sisi et al., 2011, Javorsky et al., 2012, Li et al., 2014, Song et al., 2017)	T2DM; BMI; Height; Two-hour glucose
KCNQ1	(Yasuda et al., 2008, Unoki et al., 2008, Saif-Ali et al., 2011b, Saif-Ali et al., 2011a, van Vliet-Ostaptchouk et al., 2012, Sun et al., 2012, Sun et al., 2014, Ma et al., 2015, Riobello et al., 2016)	(Aquilante, 2010, Ohshige et al., 2010, Schroner et al., 2011a, Li et al., 2017)	T2DM; Height; Serum creatinine; Fasting glucose; Serum cystatin c; HOMA-B; Insulin at 30min OGTT; Schizophrenia; Insulin at 30min OGTT adj BM; Precardial adipose tissue volume; visceral adipose tissue attenuation
HNF1α	(Gill-Carey and Hattersley, 2007, Billings and Florez, 2010, Voight et al., 2010, Estrada et al., 2014, Prasad and Groop, 2015)	(Ellard and Colclough, 2006, Pearson et al., 2000, Khelifa et al., 2016)	T2DM; Cholesterol; LDL cholesterol; Coronary artery disease; Two-huor glucose; Height; Hip circumference; HDL cholesterol
TCF7L2	(Grant et al., 2006, Hattersley, 2007, Pearson et al., 2007, Cauchi and Froguel, 2008, Gloyn et al., 2009, Villareal et al., 2010, Holstein et al., 2011, Schroner et al., 2011b, Wang et al., 2013)	(Pearson et al., 2007, Schroner et al., 2011b, Holstein et al., 2011, Song et al., 2017)	T2DM; Fasting glucose

Table 3.1: Supporting evidence for causal involvement in T2DM risk or sulphonylurea response.

A literature search was conducted to identify evidence of an association with either risk of T2DM or an effect on response to sulphonylurea. The Type 2 Diabetes Knowledge portal was also used to identify phenotypes that had evidence for signal in their datasets.

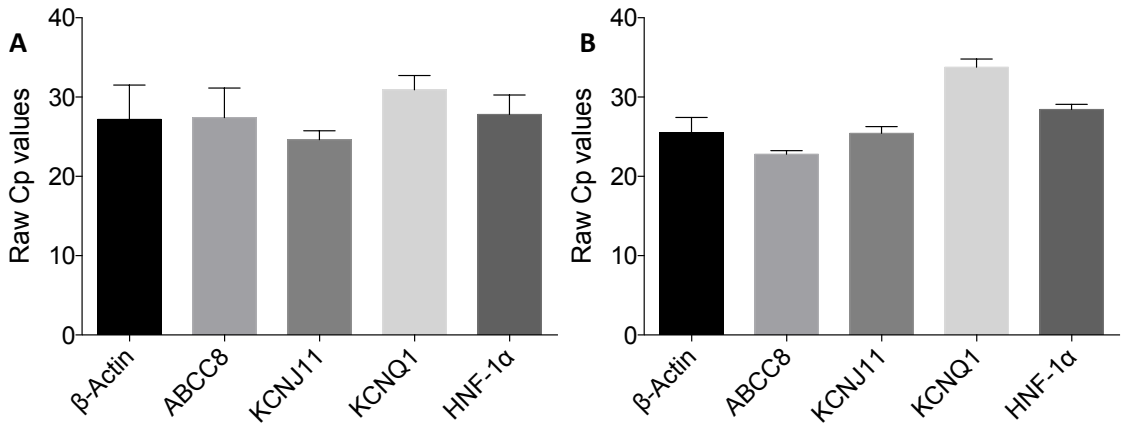


Figure 3.2: Candidate gene expression in pancreatic β-cell lines

Total RNA was extracted from (A) BRIN-BD11 and (B) MIN6 cells and reverse transcribed into cDNA. Expression of candidate genes was then determined through qPCR, with β-actin used as the house keeping gene. Data are presented as mean ± SEM (n = 3).

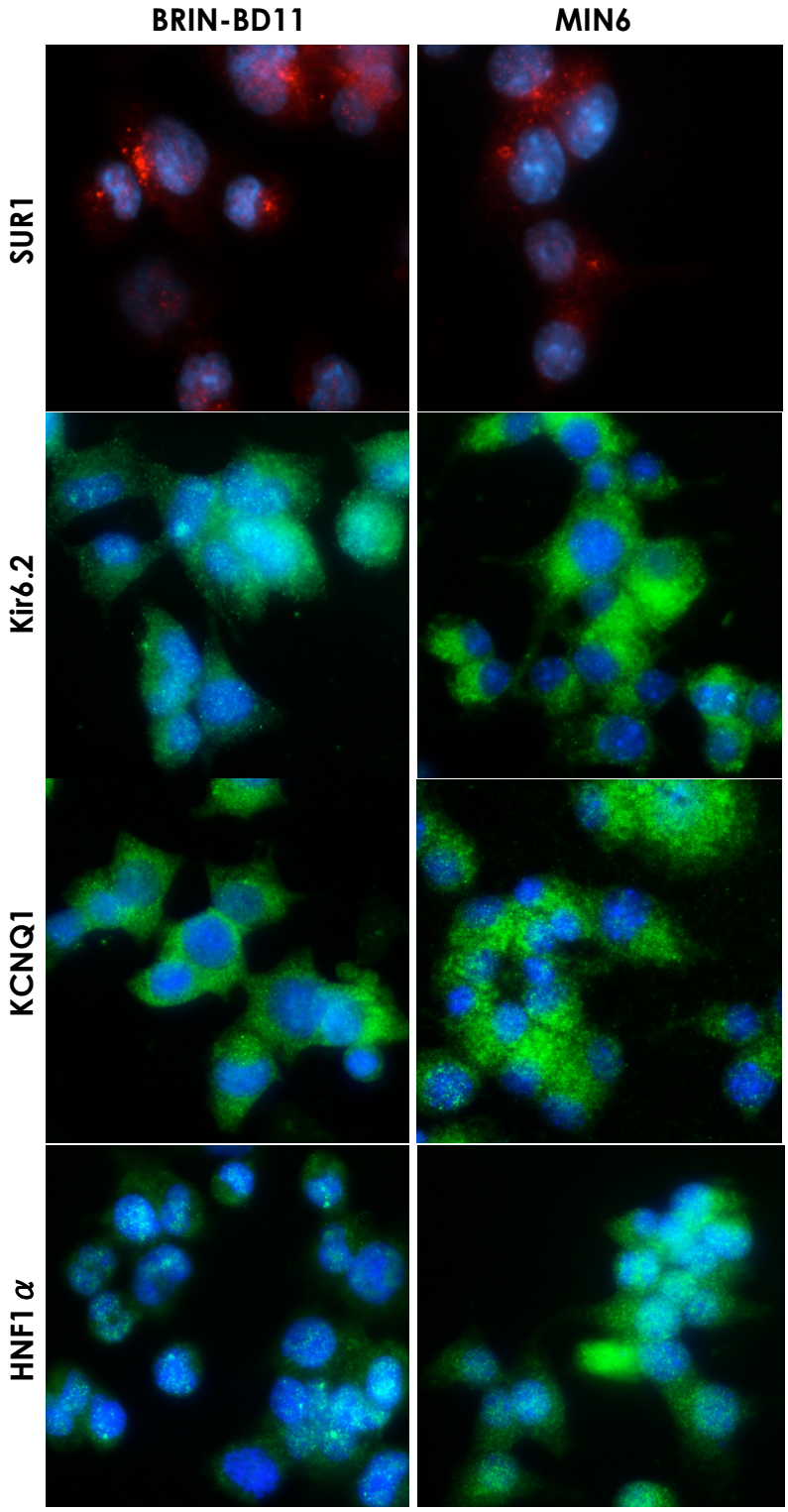


Figure 3.3: Expression of candidate biomarkers at protein level in MIN6 and BRIN-BD11 cell lines

The expression of candidate biomarkers in MIN6 cell line was confirmed by immunocytochemistry. SUR1, Kir6.2, KCNQ1 and HNF1α were expressed. Images representative of 3 independent experiments are shown.

3.3.3 Potassium channel inhibition affects sulphonylurea-induced insulin secretion

An initial assessment of the role of candidate biomarkers on sulphonylurea response was conducted using simple antibody blocking experiments. To assess the impact of cell surface potassium channel proteins on sulphonylurea-induced insulin secretion, BRIN-BD11 cells were exposed to antibodies against SUR1, Kir6.2 and KCNQ1 prior to sulphonylurea treatment. The resulting effect on sulphonylurea-induced insulin secretion was measured by ELISA and is shown in Figure 3.4. Unsurprisingly, blockage of SUR1 inhibited sulphonylurea-induced insulin secretion in response to both tolbutamide (54.63 ± 0.02% reduction, $P<0.0001$) and glibenclamide (50.53 ± 0.04% reduction, $P<0.0001$). However, blockage of Kir6.2 only inhibited insulin secretion in response to glibenclamide (49.47 ± 0.04% reduction, $P<0.0001$). This is consistent with the proposal that glibenclamide may have some affinity for Kir6.2 (Selvin and Renuad 2015). Blockage of KCNQ1 had no effect on sulphonylurea induced insulin secretion. However, KCNQ1 does not host a sulphonylurea binding site and the reported role of KCNQ1 in response to sulphonylureas may take place further downstream of K_{ATP} channel activation.

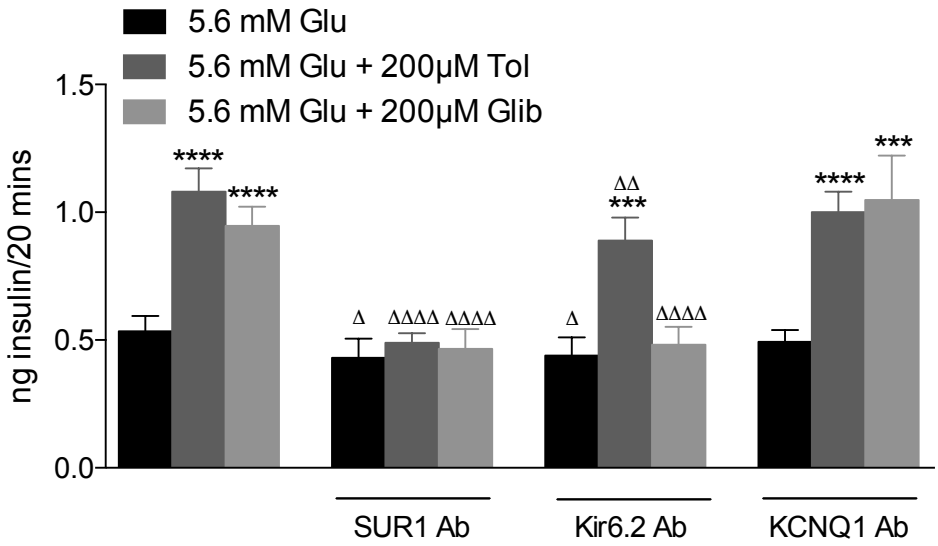


Figure 3.4: Effect of potassium channel inhibition on BRIN-BD11 response to sulphonylureas.

Following addition of 5 µg of antibody against SUR1, Kir6.2 or KCNQ1, BRIN-BD11 monolayers were incubated for 24 h. Following this, insulin secretion in response to 200 µM Tolubitamide or Glibenclamide was tested by ELISA. Data are presented as mean ± SEM. (n = 3). ***P<0.001, ****P<0.0001 compared with corresponding control (5.6 mM Glucose). $^{\Delta}P$<0.05, $^{\Delta\Delta}$ P<0.01, $^{\Delta\Delta\Delta\Delta}$ P<0.0001 compared with equivalent treatment in the absence of blocking antibody. (GLU, glucose; Tol, tolbutamide; Glib, glibenclamide)

3.3.4 siRNA transfection has no significant impact on pancreatic β-cell line viability

Concerns regarding transfection reagent induced toxicity prompted the decision to assess the impact, of rising concentrations of transfection reagent on the number of proliferating BRIN-BD11 cells. This was assessed at 3 different time points following exposure to transfection reagent using an MTT assay. As seen in Figure 3.5, at both the 24 and 48-hour time points there is no significant difference between the 3 groups. At 72 h, absorbance is higher in cells treated with the transfection reagent when compared to the control group (P<0.05) suggested that, at these concentrations, transfection reagent does not adversely affect the number of proliferating cells. Although we do see a rise in absorbance between 24 and 48 h this is most likely due to the increased number of proliferating cells. The absorbance value then falls from 48 to 72 h, this

was most likely due to cells becoming over confluent in the wells, and starting to strip off. The result confirmed that these high amounts of transfection reagents were not having an impact of BRIN-BD11 cell viability, and based on these observation 6 μl of transfection reagent was used in future transfections.

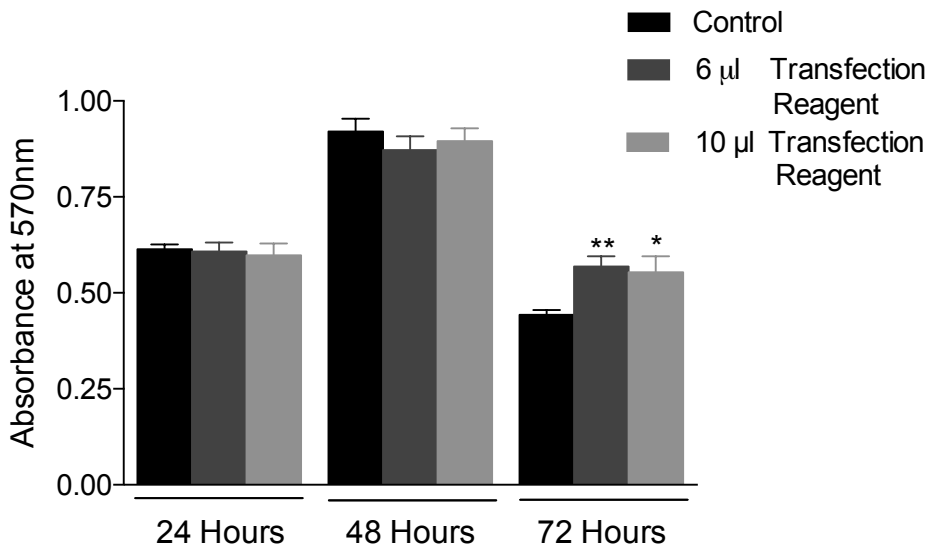


Figure 3.5: siRNA transfection has no significant impact on viability of BRIN-BD11 cells

Following addition of transfection reagent, the effect on cell viability in this cell line was studied using an MTT assay. Data are presented as mean ± SEM (n=3), *P<0.05, **P<0.01 compared with control at the corresponding time point.

3.3.6 Transfection efficiency using siRNA in pancreatic β-cell lines

The expression of candidate biomarkers was silenced in both BRIN-BD11 and MIN6 cells using 100 nm siRNA against the rat or mouse sequences (for BRIN-BD11 and MIN6 respectively) of ABCC8, KCNJ11, KCNQ1 and HNF1α. Gene silencing was quantified by qPCR as shown in Figure 3.6.

Silencing of ABCC8 in BRIN-BD11 cells was 41.98 ± 9.623% of scrambled control (P<0.005, Figure 3.6A). However, ABCC8 silencing in MIN6 cells was less efficient with expression levels post transfection 74.05 ± 12.26% of scrambled control (*NS,* Figure 3.6B).

Silencing of KCNJ11 in BRIN-BD11 cells was 40.30 ± 9.35% of scrambled control (*P*<0.005, Figure 3.6A). Consistently, KCNJ11 silencing in MIN6 cells was less efficient with expression levels post transfection at 28.57 ± 14.67% of scrambled control (*NS*, Figure 3.6B).

Silencing of KCNQ1 in BRIN-BD11 cells was 54.14 ± 5.91% of scrambled control (*P*<0.0001, Figure 3.6A). KCNQ1 silencing in MIN6 cells was comparable with BRIN-BD11 cells with expression levels post transfection at 51.26 ± 11.37% of scrambled control (*P*<0.005, Figure 3.6B).

Finally, silencing of HNF1α in BRIN-BD11 cells was 2.47 ± 1.35% of scrambled control (*NS*, Figure 3.6A). Knockdown of HNF1α was not achieved in BRIN-BD11 despite attempts to optimize four different siRNAs; therefore these experiments were excluded from further insulin secretion assays. Conversely, HNF1α silencing in MIN6 cells was more successful with expression levels post transfection at 56.74 ± 7.99% of scrambled control (*P*<0.0001, Figure 3.6B).

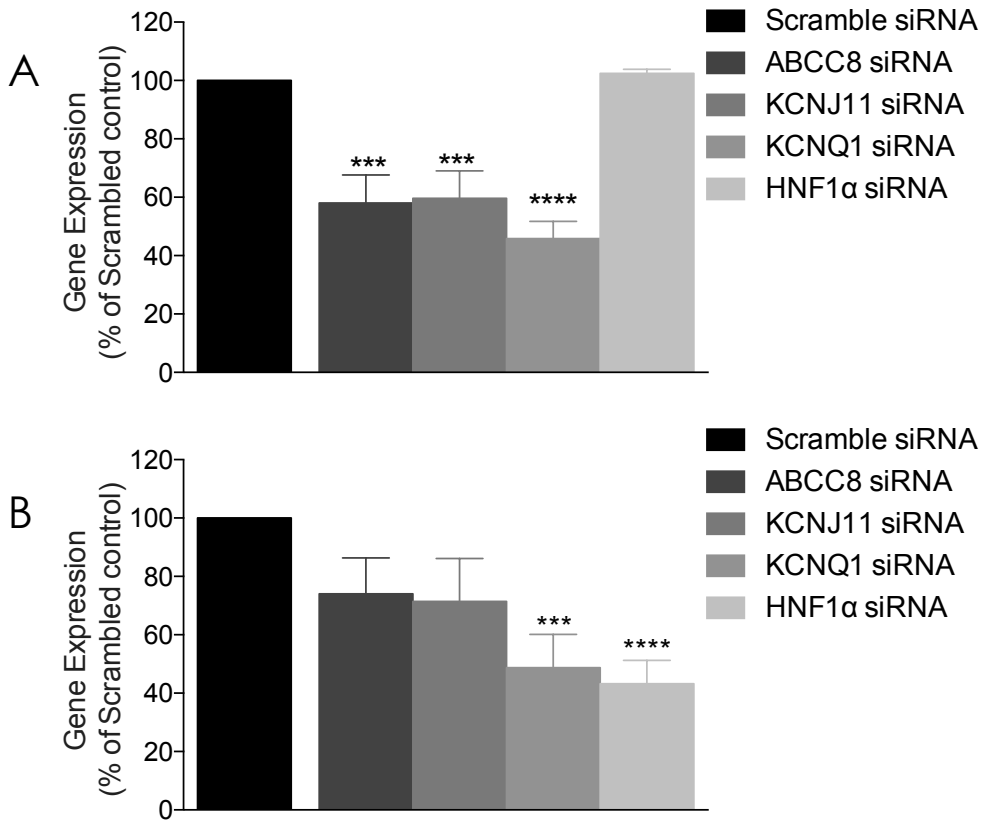


Figure 3.6: Candidate biomarker knockdown using siRNA in BRIN-BD11 and MIN6 cell lines

Candidate biomarker knockdown at the mRNA level, using 100nm siRNA, against ABCC8, KCNJ11, KCNQ1 and HNF1α in BRIN-BD11 (A) and MIN6 (B) cells was assessed using qPCR. The figure shows the percentage knockdown in comparison to the scrambled siRNA used for each cell line. Data are presented as mean ± SEM with n=7-8 for BRIN-BD11 and n=6-9 for MIN6. ***P<0.001, ****P<0.0001 compared with the corresponding scrambled siRNA.

3.3.7 ABCC8 drives sulphonylurea response in pancreatic β-cell lines

Following knockdown of expression of each candidate biomarker, the impact on insulin secretion from pancreatic β-cell lines was assessed using ELISA, and is represented as a % of control (Scrambled siRNA), in BRIN-BD11 (Figure 3.7A) and MIN6 (Figure 3.7B) cells.

Following ABCC8 knockdown in BRIN-BD11 cells (Figure 3.7A), significant reductions in insulin secretion were observed in response to 1.1 mM glucose (24.16 ± 9.87% reduction; P<0.05) and 16.7 mM glucose +

glibenclamide (49.02 ± 7.47% reduction, *P*<0.0001) when compared with the scrambled siRNA control. In the MIN6 cell line (Figure 3.7B) the subsequent effect on insulin secretion following ABCC8 knockdown was not replicated.

KCNJ11 knockdown in the BRIN-BD11 cell line (Figure 3.7A) caused a significant reduction in insulin secretion in response to 16.7 mM glucose + glibenclamide (26.08 ± 7.15% reduction, *P*<0.005) compared to scrambled control. Again, this finding was not replicated in the MIN6 cell line (Figure 3.7B).

Significant reductions in tolbutamide (29.00 ± 9.27% reduction, *P*<0.01) and glibenclamide (37.00 ± 8.86% reduction, *P*<0.005)-potentiated insulin secretion were observed in BRIN-BD11 cells, following KCNQ1 knockdown (Figure 3.7A). Consistent with observations following ABCC8 and KCNJ11 knockdown in MIN6 cells, KCNq1 knockdown did not significantly alter insulin secretion in response to any secretagogue tested (Figure 3.7B).

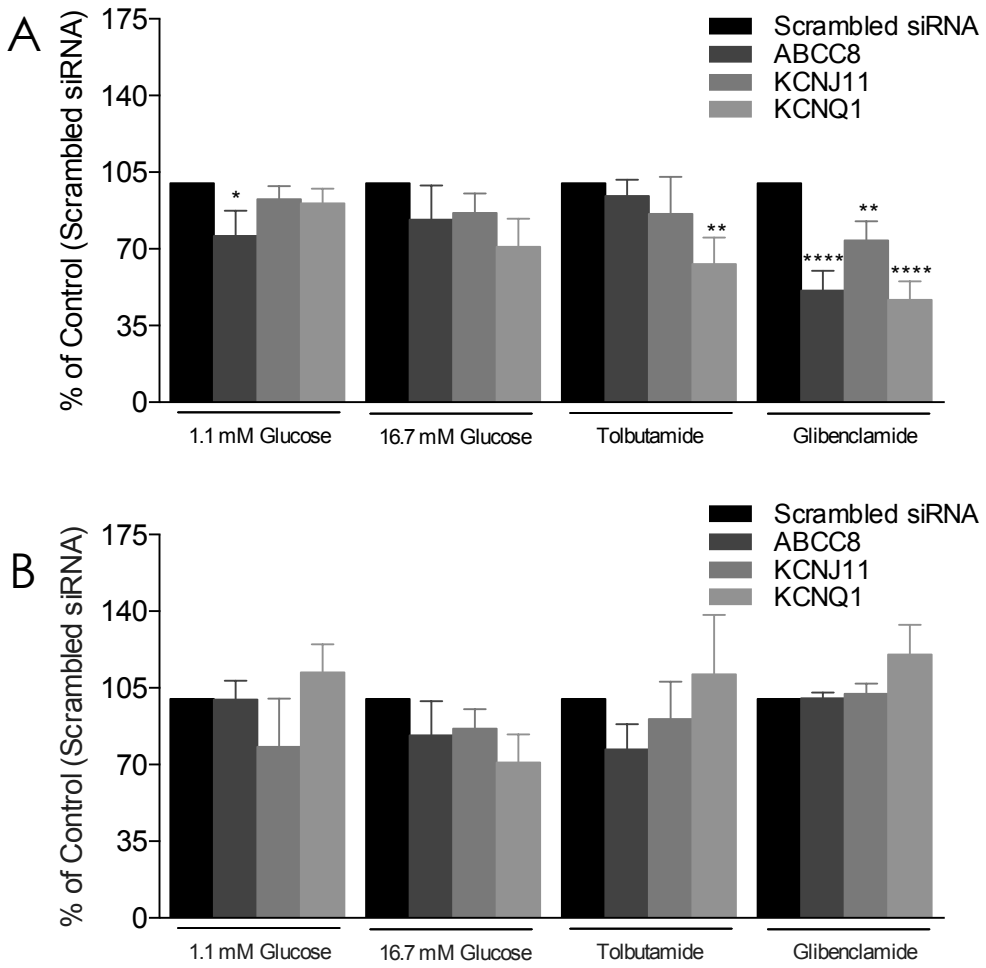


Figure 3.7: Effect of candidate biomarker knockdown on insulin secretion from BRIN-BD11 cells

Insulin secretion in response to glucose and sulphonylureas from BRIN-BD11 (A) or MIN6 (B) cells following knockdown of candidate biomarkers using 100 nm siRNA was assessed by ELISA. Tolbutamide and glibenclamide where added in combination with 16.7mM glucose. Data are presented as mean ± SEM with n=3-5 for BRIN-BD11 and n=4-6 for MIN6. *P<0.05, **P<0.01, ****P<0.0001 compared with the corresponding scrambled siRNA.

3.4 Discussion

It is known that inter-individual response to sulphonylurea treatment exists and that a genetic component is likely to play a role in varying response rates. Several genes have been highlighted as having a significant impact on sulphonylurea response including *ABCC8*, *KCNJ11*, *KCNQ1*, *HNF1α* and *TCF7L2* (Glamoclija and Jevric-Causevic, 2010, Schroner et al., 2011, Klen et al., 2014, Urbanova et al., 2015, Loganadan et al., 2016).

However, individual studies have identified different loci within each of these genes that are deemed to be important in terms of response (Pearson et al., 2000, Meirhaeghe et al., 2001, Schroner et al., 2011, Li et al., 2014). Furthermore, little consistency exists within the literature as to which SNPs are causative in sulphonylurea failure. Much of this variation may result from differences in cohort characteristics. For example, different ethnic groups have worse macrovascular complications of diabetes (Golden et al., 2012). However, there are a significant number of loci linked with increased risk to T2DM irrespective of ethnicity (Spanakis and Golden, 2013). This study therefore endeavored to investigate the role of each of these candidate genes on sulphonylurea response from single β–cells in an effort to better understand the mechanisms behind sulphonylurea response.

Two β–cell lines were chosen for the study in an attempt to ensure that any observed changes in sulphonylurea response did not result from the nuances of one particular cell line. Additionally, first and second generation sulphonylureas (tolbutamide and glibenclamide respectively) were employed to determine if drug chemistry had an effect on response. The BRIN-BD11 and MIN6 cell lines have previously been shown to be sulphonylurea responsive (Brennan et al., 2006, Morioka et al., 2012), which was again confirmed in the current study where significant enhancements in sulphonylurea-induced insulin secretion were observed. Furthermore, the cell lines were found to express four of the five candidate genes of interest, namely *ABCC8*, *KCNJ11*, *KCNQ1* and *HNF1*α consistent with reports in the literature (Blumenfeld et al., 1991, Gloyn et al., 2006, Yamagata et al., 2011). TCF7L2 expression was not observed in either cell line in this study contrary to some reports in the literature (Migliorini and Lickert, 2015, Dong et al., 2016). It is possible that *TCF7L2* is expressed at low levels in these cell lines and that this accounts for the lack of determination of mRNA expression in this study. However, having employed numerous species-specific and primer-specific probes to examine expression, we excluded *TCF7L2* from further study.

The potassium sensitive K_{ATP} channel comprises two subunits, SUR1 and Kir6.2. *ABCC8* encodes the pancreatic β–cell surface protein SUR1, a key component of the K_{ATP} channel, and also the direct target for sulphonylureas. The effect of SUR1 inhibition was assessed following exposure to an antibody against SUR1. Unsurprisingly, the blockage of SUR1 prevented sulphonylurea binding, inhibiting subsequent downstream effects and hampering insulin release from the BRIN-BD11 pancreatic β–cell line. Following this *ABCC8* specific siRNA were used in transfections of both pancreatic β–cell lines. A clear and obvious effect on sulphonylurea-induced insulin secretion was observed in the BRIN-BD11 cell line after *ABCC8* silencing especially in response to glibenclamide. However, a significant effect was not observed in the MIN6 cell line following *ABCC8* silencing. This discrepancy may be explained by the poor transfection efficiency observed in the MIN6 cell line, which proved difficult to transfect in all instances in this study.

The second subunit of the K_{ATP} channel is the inwardly rectifying Kir6.2, encoded by *KCNJ11*. Kir6.2 is also a target of sulphonylurea action especially for second-generation drugs like glibenclamide (Kuhner et al., 2012, Proks et al., 2014). Following exposure of the BRIN-BD11 cell line to an antibody against Kir6.2, sulphonylurea-induced insulin secretion was impacted, but only in response to glibenclamide. Glibenclamide is known to carry some affinity for the Kir6.2 subunit of the K_{ATP} channel that is not associated with tolbutamide (Kuhner et al., 2012). This was supported by experiments in which *KCNJ11* was silenced in the BRIN-BD11 cells. As with the antibody blocking experiment, glibenclamide-, but not tolbutamide-induced insulin secretion was significantly impaired. As mentioned above, glibenclamide does carry affinity for the Kir6.2 subunit, and given that second generation sulphonylureas are typically more potent than first (tolbutamide) this result is perhaps not surprising (Aquilante, 2010). Consistently, a significant effect in sulphonylurea-induced insulin secretion was not observed following attempts to silence

KCNJ11 in the MIN6 cell line, likely reflecting the poor transfection efficiency in this model.

KCNQ1 encodes a voltage-gated K^+ channel in the pancreatic β–cell. As a cell surface protein, the effect on sulphonylurea-induced insulin secretion following exposure to KCNQ1 antibody was assessed, prior to undertaking siRNA transfections in the cell line models. Following antibody blocking of the channel, no impact on sulphonylurea-induced insulin secretion from BRIN-BD11 cells was observed. However, this was not a result replicated following transfection with *KCNQ1* specific siRNA where highly significant reductions in both tolbutamide- and glibenclamide-induced insulin secretion were observed. The antibody blocking experiments hamper channel activity by occupying the binding site on *KCNQ1*. However, it is possible that the effect on sulphonylurea-induced insulin secretion observed in the BRIN-BD11 cell line following *KCNQ1* knockdown highlights an important role for *KCNQ1* expression and not just channel activity in the regulation of this process. Once again, poor transfection efficiency in the MIN6 cell line was observed and attempts at *KCNQ1* silencing were associated with a limited effect on insulin secretion in this instance. Yamagata et al. (2011) showed that overexpression of *KCNQ1* could regulate insulin secretion in the MIN6 β–cell line, with insulin secretion in response to tolbutamide significantly impaired. It was proposed that the increase in channel density lowered the rate of action potentials and decreased insulin release from the β–cell. Collectively, *KCNQ1* appears to play an important role in insulin secretion from the pancreatic β–cell, which warrants further investigation.

Prior work has shown that mutations in *HNF1α* causes sensitivity to sulphonylureas (Ashcroft and Rorsman, 2012). As HNF1α is not a cell surface protein it was not possible to conduct an antibody blocking experiment in this study. However, multiple attempts were made to assess the effect of *HNF1α* deficiency on sulphonylurea-induced insulin

secretion in the BRIN-BD11 and MIN6 cell lines. Despite optimization of four independent siRNAs against HNF1α, knockdown at the gene level was not achieved in the BRIN-BD11 cell line. Although good transfection efficiency was achieved in the MIN6 cell line, a limited effect on insulin secretion was observed.

To summarise, while transfection efficiency was better in the BRIN-BD11 cell line compared with the MIN6 cell line. In the BRIN-BD11 cell line, *ABCC8*, *KCNJ11* and *KCNQ1* all significantly impaired insulin secretion in response to glibenclamide. Our data suggests that *ABCC8*, *KCNJ11*, and *KCNQ1* may have a role to play in the direct control of SU-driven insulin secretion suggesting that genes beyond the K_{ATP} channel are important in response to this drug class.

Chapter 4:

Demographics of the DIASTRAT cohort

4.1 Introduction

Treatment of T2DM involves changes in lifestyle and pharmacological treatment (National Institute for Health and Care Excellence (NICE), 2015). There is typically a focus on educating the patients about their disease and how to self manage the disease along with dietary advice (National Institute for Health and Care Excellence (NICE), 2015). Priorities thereafter involve blood pressure management and blood glucose management, with specific targets for each (140/80 mmHg and 48 mmol/mol respectively) (National Institute for Health and Care Excellence (NICE), 2015). If lifestyle and dietary changes are insufficient to meet these targets then pharmacological interventions are indicated (National Institute for Health and Care Excellence (NICE), 2015).

Diabetes control is monitored by measuring a patient's glycated haemoglobin (HbA_{1c}), giving an insight into glycaemic control over the previous two to three months (Sherwani et al., 2016). As part of normal physiological function, haemoglobin is regularly glycated, with the most abundant fraction formed being HbA_{1c} (Sherwani et al., 2016). The amount of circulating HbA_{1c} is directly proportional to the amount of ambient blood glucose; therefore, as blood glucose increases, so too does HbA_{1c} (Sherwani et al., 2016). As the lifespan of an erythrocyte is typically two to three months, when HbA1c is measured it gives an insight into how well the patient's glycaemia has been controlled over that time period (Sherwani et al., 2016). In the diabetic population, HbA_{1c} should be routinely monitored every 3-6 months (National Institute for Health and Care Excellence (NICE), 2015). The target set by the NICE guidelines for good glycaemic control is 48 mmol/mol. If the first line pharmacological therapy, metformin, is not tolerated, or if HbA_{1c} does not fall below 58 mmol/mol, additional therapies will be introduced. Amongst these is the sulphonylurea drug class, which acts to enhance insulin secretion from the pancreatic β–cells. This drug class is associated

with poor response rates and will be the primary focus of the current study,

Given the complex aetiology of the disease, the T2DM population is notoriously heterogonous in terms of clinical characteristics and response to treatment. Much of the variation relates to metabolic capabilities, socioeconomic differences, environmental influences, ethnicity and differences in severity of disease (Cantrell et al., 2010). Response to therapy is further complicated by the significant level of polypharmacy observed in the T2DM population (Noale et al., 2016). Polypharmacy describes the phenomenon of multiple medications prescribed concurrently, and typically increases with age (Peron et al., 2015), in T2DM it's not uncommon to see patients with more than one anti-diabetic treatment, for example an SU and INS concurrently, with perhaps a statin for stating to combat high cholesterol too. Polypharmacy-related complications typically arise due to increased risk of nonadherence to drug, drug-drug interactions and adverse drug events (Peron et al., 2015). Among older patients with T2DM the factors that contribute to polypharmacy include micro- and macro-vascular complications including diabetic nephropathy and retinopathy, coronary artery disease, geriatric syndromes and cognitive impairment (Peron et al., 2015).

Given the above, thorough characterization of cohort characteristics and clinical information (to include prescribing information) are an important part of any cohort study in the T2DM population. As such, the aims were clear - to firstly characterise participant demographics within a T2DM cohort (DIASTRAT) recruited from the Western Health and Social Care Trust through collecting clinical, anthropometric and biochemical information. To understand the prevalence of polypharmacy among the cohort. To garner an initial impression of glycaemic control within the cohort through studying HbA_{1c} levels and finally, to assess plasma c-peptide and insulin levels as an indicator of residual β–cell function.

4.2 Materials and Methods

4.2.1 Chemicals

Reagents of analytical grade and deionized water were used. All other chemicals used are listed in Chapter 2, Section 2.1.

4.2.2 Participant Recruitment

Participants were recruited onto the Stratified Medicine, optimizing treatment for Diabetes (DIASTRAT) study as described in Chapter 2, Section 2.2.15.

4.2.3 Database Construction

Participant clinical, anthropometric and biochemical information was collated into a database, full detail are provided in Chapter 2, Section 2.2.21

4.2.4 Processing blood samples

Blood samples taken from consented participants was processed using the method described in Chapter 2, Section 2.2.16.

4.2.5 Plasma insulin levels

Participant plasma insulin levels were assessed using the Mercodia Human Insulin ELISA kit as described in Chapter 2, Section 2.2.19.

4.2.6 Plasma C-peptide levels

Participant plasma c-peptide levels were assessed using the ALPCO C-peptide ELISA, the method used is described fully in Chapter 2, Section 2.2.20.

4.2.7 Statistics

Data are presented as mean ± SD for a given number of participants (N). Comparisons of clinical, anthropometric and biochemical data between subgroupings were assessed by One-way ANOVA with post-hoc test.

Significant correlations between HbA1c, or c-peptide (as markers of glycaemic control and residual β–cell function respectively) and other characteristics, were identified using Spearman's coefficient (*p*). Significance was accepted at $P<0.05$.

4.3 Results

4.3.1 Baseline characteristics of the DIASTRAT cohort

Demographic information on the cohort is collated in Table 4.1. Of the 500 participants recruited, 10 were eventually excluded for exceeding the maximum age range of the cohort (18 – 80 years). The cohort was predominantly male (63%) with a mean age of 63 (± 11.12) years. Mean weight was 96.08 (± 20.70) kg, BMI was 33.93 (± 7.77) and height was 168.50 (± 10.30) cm. Where data was available, the average alcohol consumption for the cohort was 0.36 (± 1.06) units/day. However, the data for alcohol consumption is unlikely to be fully representative of the cohort as a whole, as alcohol consumption data was recorded for just 57% of all participants, with the majority of those recording zero alcohol consumption.

For analyses, the cohort was sub-grouped based on their use of exogenous insulin (INS) or sulphonylureas (SU). Significant differences ($P<0.05$) in weight and BMI were observed between those received INS in the absence of SU and those receiving SU in the absence of INS (Figure 4.1). In both instances, values for those in the SU^+/INS^- group (weight: 100.10 ± 22.24 kg; BMI: 34.85 ± 7.70) were significantly higher than those in the SU^-/INS^+ grouping (weight: 94.07 ± 19.82 kg; BMI: 32.97 ± 6.66).

Chapter 5:

Protein signature of the DIASTRAT cohort

5.1 Introduction

Inflammation is the biological process responsible for protecting against pathogens and controlling repair and healing after cell damage (Kraakman et al., 2014). If inflammation is not contained following an acute exacerbation, the continuous activation of the immune system can trigger tissue damage and disease (Kraakman et al., 2014). With both obesity and T2DM there is evidence of persistent low-grade inflammation, with a rise in circulating inflammatory cytokines and acute-phase proteins (Touch et al., 2017). In obese individuals this low-grade inflammation contributes to insulin resistance, the main pathological change that links both obesity and T2DM (Zhao et al., 2014). The low grade inflammation typically seen in obesity and T2DM is characterised by an increase in circulating TNF-α, as well as inflammatory mediators like C-reactive protein (CRP), serum amyloid A (SAA), the pro-inflammatory cytokine interleukin-6 (IL-6), and the chemokine's IL-8, C-C chemokine ligand 2 (CCL2)/monocyte chemoattractant protein-1 (MCP-1) and CCL5/RANTES (regulated on activation, normal T cell expressed and secreted). However, the circulating levels of all of these proteins can be reduced following weight loss (Hotamisligil et al., 1995, Dalmas et al., 2011). It has also been reported that increased interleukin-1β (IL-1 β), IL-6 and CRP is predictive of T2DM (Pradhan et al., 2001, Spranger et al., 2003, Donath and Shoelson, 2011).

Tumor necrosis factor-alpha (TNF-α) can facilitate insulin resistance in obesity and diabetes, it does this, in part, by inhibiting the tyrosine kinase activity of the insulin receptor (Schenk et al., 2008). The inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and c-Jun N-terminal kinases (JNK) can also enhance obesity associated insulin resistance (Eguchi and Manabe, 2013). Obesity can also change cytokine secretion from adipose tissue and modify gene expression in adipocytes causing activation of inflammatory processes within the

adipose tissue (Eguchi and Manabe, 2013). In those who are obese, adipose tissue will secrete lower amounts of anti-inflammatory adipokines and higher amounts of proinflammatory cytokines, like IL-6 and TNF-α (Eguchi and Manabe, 2013). Also, due to activated lipolysis in obesity, free fatty acids are released, causing the activation of inflammatory signalling and the release of proinflammatory mediators; this response provides the link between obesity and insulin resistance (Eguchi and Manabe, 2013). Adipose tissue also contains macrophages, which produce a large amount of pro-inflammatory factors that are upregulated in obesity. The number of macrophages increases with increasing obesity, and alters the activation status of the cells (Donath and Shoelson, 2011).

Those with T2DM can also present with histological changes in the pancreas, in the form of amyloid deposits (Westermark, 1972), immune cell intrusion (Ehses et al., 2007) or cell death/fibrosis (Yoon et al., 2003), in the pancreatic islets perhaps caused by inflammation (Eguchi and Manabe, 2013). This is perhaps indicative of a role for inflammation in the progress of pancreatic β-cell dysfunction (Eguchi and Manabe, 2013). Inflammation also plays a role in atherosclerotic plaque formation, a key step in atherosclerosis, which is instrumental in the progression of cardiovascular disease – a common comorbidity seen in T2DM (Martin-Timon et al., 2014, Golia et al., 2014)

Despite the body of evidence in support of a role for inflammation in the progression from obesity to diabetes, a unique protein signature associated with the diabetic state is not well defined. Therefore, this study sought to examine the inflammatory profile within a T2DM cohort (DIASTRAT) using large-scale screens for proteins associated with inflammation and cardiovascular outcomes, with a view to determining if any such marker may be predictive of glycaemic control within this population.

5.2 Materials and Methods

5.2.1 Chemicals

Reagents of analytical grade and deionized water were used. All other chemicals used are listed in Chapter 2, Section 2.1.

5.2.2 Participant Recruitment

Participants were recruited onto the Stratified Medicine, optimizing treatment for Diabetes (DIASTRAT) study as described in Chapter 2, Section 2.2.15.

5.2.3 Processing Blood samples

Blood samples taken from consented participants was processed using the method described in Chapter 2, Section 2.2.16.

5.2.4 Proximity Extension Assay (PEA)

Plasma samples were used for PEA. This work was outsourced to Olink Bioscience, Uppsala, Sweden. Details of this are described in Chapter 2, Section 2.2.20

5.2.5 Statistics

Comparison of protein marker expression between those with T2DM and apparently healthy controls was conducted using unequal t tests as described in detail in Chapter 2, Section 2.2.22. Dr Steven Watterson (Ulster University) wrote the scripts used to undertake this analysis. Comparison of protein expression within subgroupings of the T2DM cohort was conducted by one-way ANOVA with posthoc test. Data are presented as mean ± min/max. Pearson's correlation of normalized expression data with clinical parameters was conducted on the entire cohort ('All participants') or those receiving a sulphonylurea ('SU+'). In all instances, significance was accepted at $P<0.05$.

5.3 Results

Olink proximity assays were conducted on the following 4 panels: Cardiovascular II panel, Cardiovascular III panel, Immune response panel and the Inflammation panel. Each panel comprised 92 proteins leading to an initial analysis of 368 proteins across the 4 panels. These panels contained a small number of proteins repeated across multiple panels (i.e. IL-6 is present on 3 of the 4 panels), which has been ignored for initial analysis and thereafter removed from the results data.

Initially, the data comprised 374 patients with diabetes and 20 control participants, each of whom had measurements taken across 368 proteins. Any patient sample that failed QC, this QC value is set by Olink, was removed from the analyses. Similarly, several proteins across all four panels fell below the level of meaningful detection and have been excluded from the analyses for all participants. Following these exclusions, the cohort consisted of 20 control samples, 354 samples from people with diabetes and measurements across 292 proteins.

5.3.1 Identification of a protein signature indicative of a T2DM phenotype in the DIASTRAT cohort

The aim of this analysis was to generate a formula that would yield a single number for each participant that can be applied to a threshold to robustly identify the cohort to which they belong.

To this end, differential expression of all 292 detectable proteins was evaluated between cohorts using an unequal *t*-test and those with p-values below 0.05 were highlighted. This yielded 75 proteins in which significant differential expression was observed (Table 5.1).

Protein	P value	Protein	P value
KLRD1	0.0001	HAOX1	0.0140
KIM1	0.0004	t-PA	0.0151
TNFRSF14	0.0005	REN	0.0163
FGF-21	0.0005	GLB1	0.0178
EGLN1	0.0006	LILRB4	0.0183
MMP7	0.0009	PRDX5	0.0194
CD84	0.0009	VSIG2	0.0194
CCL4	0.0009	CLEC4D	0.0204
AGRP	0.0010	CCL17	0.0214
DCBLD2	0.0011	ITGB2	0.0216
CLEC4A	0.0016	LAP TGF-beta-1	0.0230
FGF-23	0.0017	LIF-R	0.0238
FGF-23	0.0025	TR	0.0239
U-Par	0.0028	MMP12	0.0243
TNFRSF9	0.0030	HGF	0.0251
FGF-21	0.0032	IL-18R1	0.0266
IL-4RA	0.0033	PAR-1	0.0272
Gal-4	0.0034	TNFRSF11A	0.0287
LPL	0.0035	FCRL6	0.0296
TRAIL-R2	0.0041	TNFR1	0.0298
PD-L1	0.0045	CKAP4	0.0313
CTSL1	0.0047	TFF3	0.0321
SORT1	0.0053	PCSK9	0.0325
HSP 27	0.0059	CD83	0.0336
PRSS8	0.0070	VEGFA	0.0343
PA1	0.0073	CXCL6	0.0361
HO-1	0.0075	JAM-A	0.0366
HB-EGF	0.0085	CDCP1	0.0399
CLEC6A	0.0090	SLAMF1	0.0401
TNFSF14	0.0093	CASP-3	0.0402
IDUA	0.0097	ANG-1	0.0429
AREG	0.0097	TNFSF13B	0.0434
CCL3	0.0108	MMP-9	0.0443
NCR1	0.0109	PON3	0.0448
IL-18	0.0117	PDGF subunit A	0.0452
BTN3A2	0.0120	IL-1RT1	0.0470
TREM1	0.0130	AXL	0.0479
ITGA11	0.0133	IL7	0.0500

Table 5.1 Proteins with significant differential expression from healthy controls.

Proteins that were significantly altered in participants with T2DM when compared with the control cohort are listed above. Significance was accepted at $P<0.05$ following an unequal t-test.

Combination of these 75 proteins correctly identified those with T2DM to within 99.2% accuracy (3 misclassified participants; Table 5.2A). To increase accuracy to 100%, additional proteins would be required to

strengthen the signature. However, in the DIASTRAT cohort, only 75 proteins displayed differential expression when a *P* value of 0.05 was applied. To attempt to enhance accuracy, the selection cut-off was relaxed to include a *P* value of less than 0.09. This yielded 100 proteins that were differentially expressed between those with T2DM and the control cohort. However, an improvement in the accuracy of classification was not observed (Table 5.2B).

A.

Number of proteins	Number of misclassified participants	% participants correctly identified
25	37	90.1
50	10	97.3
75	3	99.2

B.

Number of proteins	Number of misclassified participants	% participants correctly identified
100	3	99.2

Table 5.2 Identification of a T2DM phenotype based on protein signature.

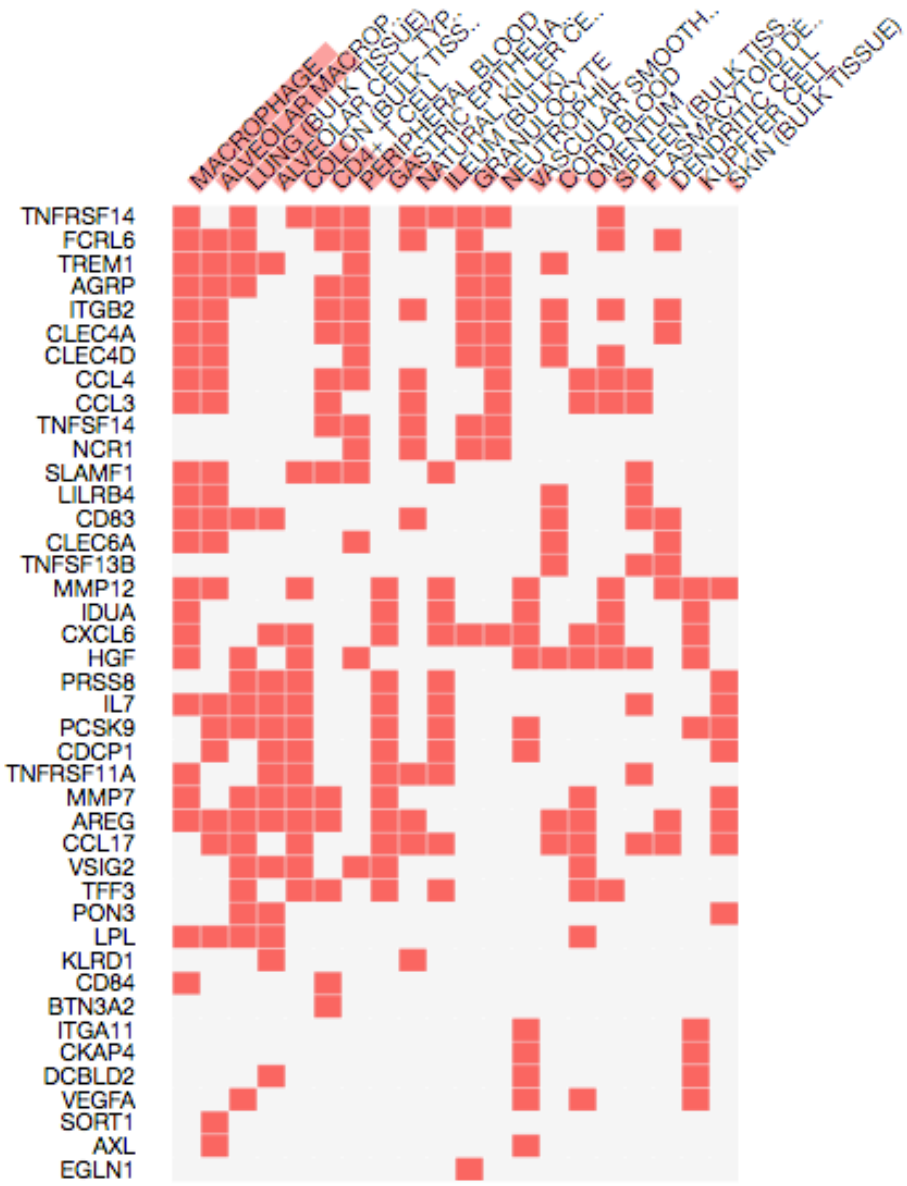
(A) Application of a *P* value threshold of 0.05 lead to the identification of a 75 protein signature that could accurately identify someone with T2DM to within 99.2% accuracy. **(B)** Relaxing the *P* value threshold to 0.09 increased the number of proteins included in the signature to 100, but did not improve accuracy.

5.3.2 Macrophages drive the inflammatory response in the DIASTRAT cohort

The 75 proteins that displayed significant differential expression between T2DM participants and those in the control cohort were further analysed to understand the ontology of cell types and tissues involved.

Gene names were entered into the Enrichr database (Chen et al., 2013, Kuleshov et al., 2016) and significant associations with cell types examined based on data from the All RNA-seq and CHIP-seq Sample Search Space (ARCHS4). ARCHS4 mines RNA-seq and CHIP-seq datasets available on the Gene Expression Omnibus (GEO). The programme analyses the aligned reads mapped to genes with counts, as well as a heatmap visualization summary of the expression data from the processed samples using Clustergrammer.

As shown in Figure 5.1, the dominant cell type involved in the inflammatory and metabolic protein signature of the DIASTRAT cohort is the macrophage (P=0.0005). Interestingly, CD4+ T cells, which in addition to macrophages are a primary driver of the T1DM phenotype, were ranked 6th in this analysis (P=0.6).



Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	MACROPHAGE	0.000005923	0.0005923	-1.54	18.55
2	ALVEOLAR MACROPHAGE	0.00006746	0.003373	-1.84	17.64
3	LUNG (BULK TISSUE)	0.004075	0.1358	-1.55	8.51
4	ALVEOLAR CELL TYPE II	0.02109	0.4217	-1.59	6.14
5	COLON (BULK TISSUE)	0.02109	0.4217	-1.42	5.48
6	CD4+ T CELL	0.04307	0.6152	-1.53	4.80
7	PERIPHERAL BLOOD	0.04307	0.6152	-1.48	4.65
8	GASTRIC EPITHELIAL CELL	0.08167	0.9999	-1.51	3.77
9	NATURAL KILLER CELLS	0.1436	0.9999	-1.37	2.65
10	ILEUM (BULK)	0.1436	0.9999	-1.27	2.46

Figure 5.1 Cell types associated with proteins highly expressed in the DIASTRAT cohort

Cell types are ranked based on outputs from the All RNA-seq and CHIP-seq Sample Search Space (ARCHS4). Data are depicted as a heatmap (top panel) and ranked according to significance (lower panel).

In addition to examining the proteins most highly expressed in the DIASTRAT cohort, examination of those poorly expressed (i.e. those that fell below the level of meaningful detection in the Olink proximity assay) was also undertaken, to determine what cell types were not involved in the inflammatory and metabolic profiles of this cohort.

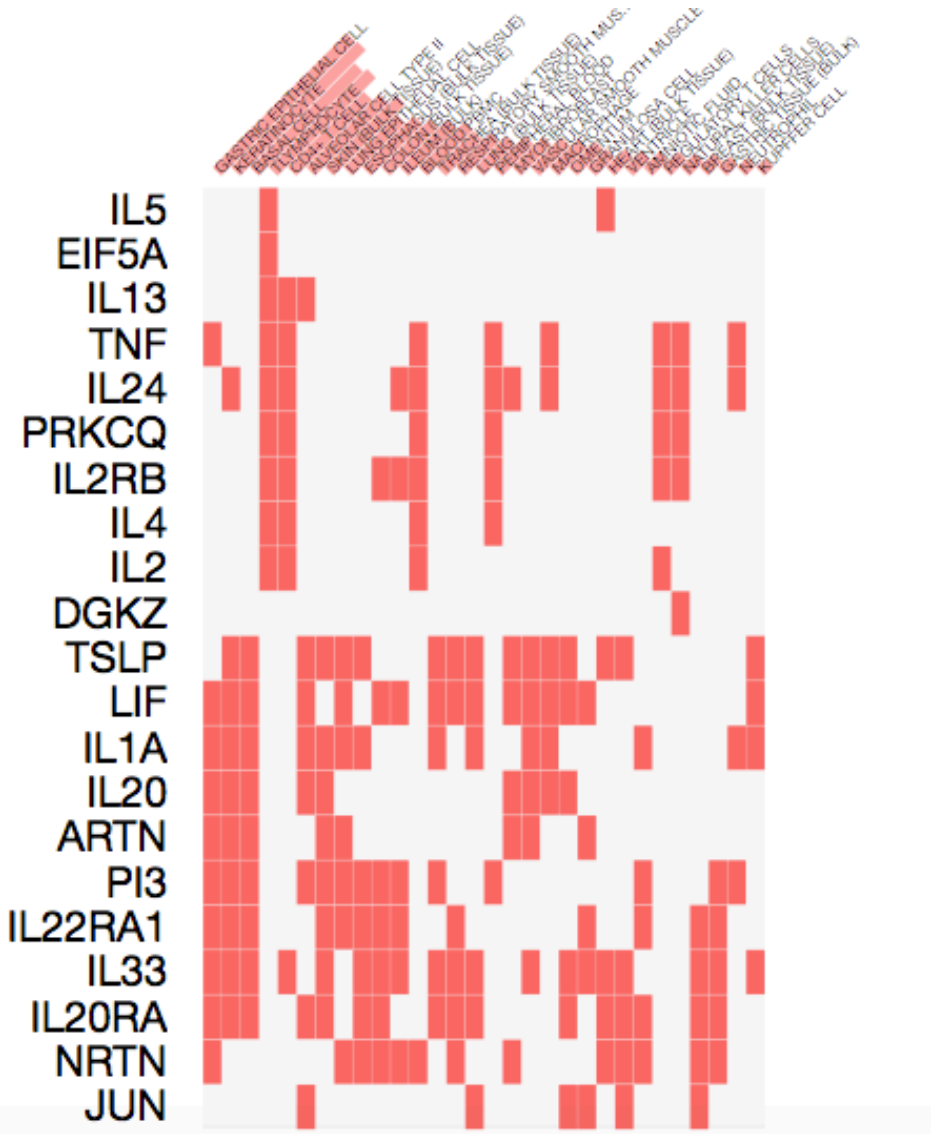
To this end, all proteins that fell below the level of detection in more than 60% of the DIASTRAT cohort were identified and the 25 proteins meeting this criterion are summarized in table 5.3. Unfortunately, because these proteins fell below the detection limits of the assay, an assessment of the abundance of each protein cannot be made and therefore, a ranking is not possible.

IL-5	IL-13	ARNT
NRTN	IL-24	PRKCQ
LIF	IL-22RA1	JUN
IL-1	TSLP	NF2
IFN-gamma	IL-2	EIF5A
IL-33	IL-1alpha	DGKZ
IL-20	IL-2RB	PI3
TNF	IL-20RA	
ARTN	KPNA1	

Table 5.3 Proteins below the detection limits of the Olink proximity assays

25 proteins were found to be below the detection limits of the Olink proximity assay in 60% or more of the cohort.

The 25 proteins not detected in this analysis are most commonly associated with gastric epithelial cells (*P*=0.01), keratinocytes (*P*=0.01) and basal cells (*P*=0.03) consistent with a non-diabetic phenotype (Figure 5.2). Interestingly, T cells and specifically, CD4+ T cells were significantly associated with this protein signature (*P*=0.07 in both instances; Figure 5.2). These findings reinforce the notion that T cells are not the primary cell type driving the inflammatory response in this cohort.



Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	GASTRIC EPITHELIAL CELL	0.0002677	0.01272	-1.70	13.99
2	KERATINOCYTE	0.0002677	0.01272	-1.66	13.66
3	BASAL CELL	0.001328	0.03153	-1.68	11.10
4	TLYMPHOCYTE	0.001328	0.03153	-1.58	10.46
5	CD4+ T CELL	0.005623	0.07631	-1.56	8.07
6	ALVEOLAR CELL TYPE II	0.005623	0.07631	-1.50	7.77
7	SKIN (BULK TISSUE)	0.005623	0.07631	-1.47	7.61
8	ESOPHAGUS (BULK TISSUE)	0.02023	0.1602	-1.47	5.73
9	LUNG EPITHELIAL CELL	0.02023	0.1602	-1.44	5.62
10	ILEUM (BULK)	0.02023	0.1602	-1.33	5.20

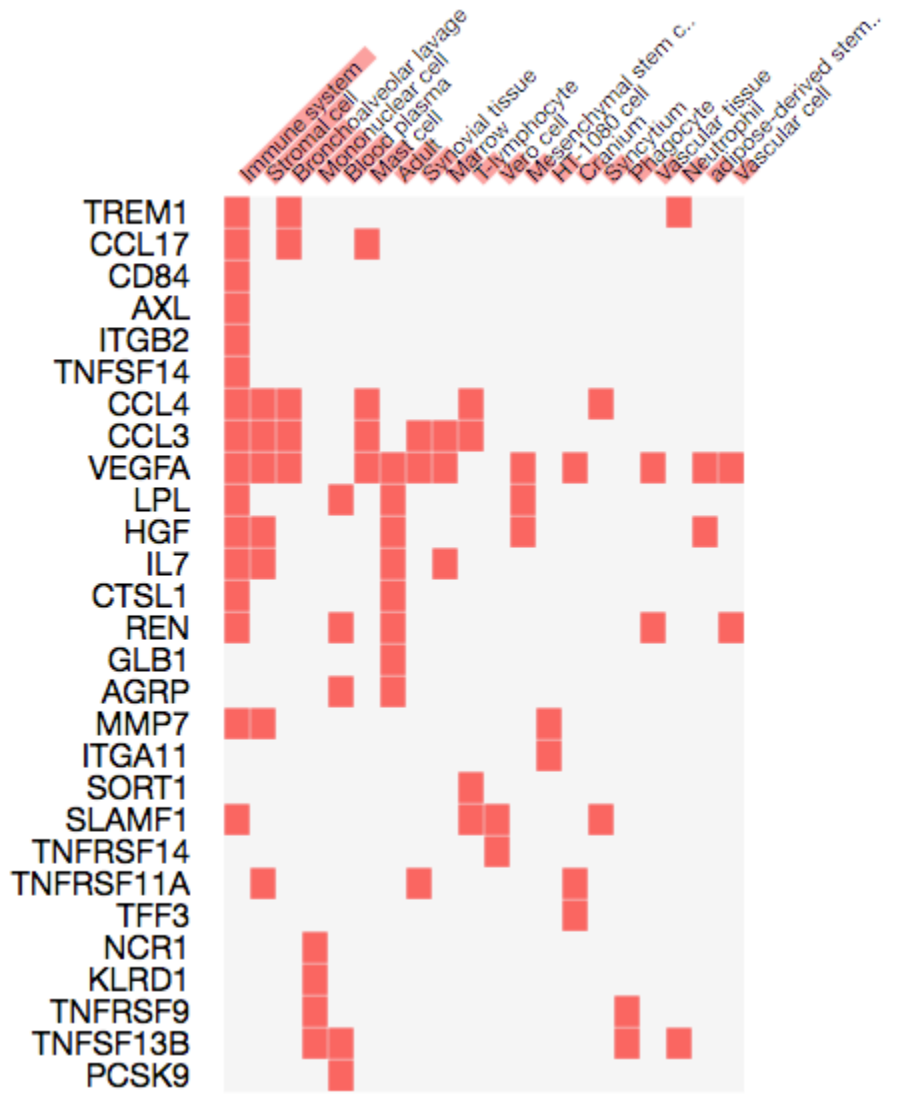
Figure 5.2 Cell types associated with proteins poorly expressed in the DIASTRAT cohort

Cell types are ranked based on outputs from the All RNA-seq and CHIP-seq Sample Search Space (ARCHS4). Data are depicted as a heatmap (top panel) and ranked according to significance (lower panel).

5.3.3 The protein signature of the DIASTRAT cohort is predominantly associated with cells of the immune system

The association of the 75 protein signature with tissue types was assessed using the JENSEN TISSUES database (Chen et al., 2013, Kuleshov et al., 2016). This database incorporates evidence on the tissue-specific expression of genes, proteomics and transcriptomics screens, and automatic text mining. The evidence is mapped to common protein identifiers and Brenda Tissue Ontology terms, and further supported through the assignment of confidence scores.

Consistent with the finding that macrophages drive the inflammatory response, the most common tissue type associated with the protein signature of the DIASTRAT cohort was cells of the immune system (P=0.0002; Figure 5.3). Interestingly, bronchoalveolar lavage was also significantly associated with this phenotype (P=0.002) consistent with the observation that alveolar macrophages played a dominant role in the inflammatory response (Figure 5.1).



Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	Immune_system	0.000001070	0.0002931	-6.92	95.10
2	Stromal_cell	0.000004956	0.0006789	-4.79	58.53
3	Bronchoalveolar_lavage	0.00003463	0.002372	-3.98	40.84
4	Mononuclear_cell	0.00001737	0.001587	-3.05	33.42
5	Blood_plasma	0.0004917	0.02246	-3.87	29.50
6	Mast_cell	0.002561	0.09892	-4.38	26.12
7	Adult	0.01988	0.1873	-6.50	25.45
8	Synovial_tissue	0.003738	0.1024	-3.66	20.44
9	Marrow	0.005750	0.1070	-3.85	19.88
10	T-lymphocyte	0.02014	0.1873	-4.26	16.63

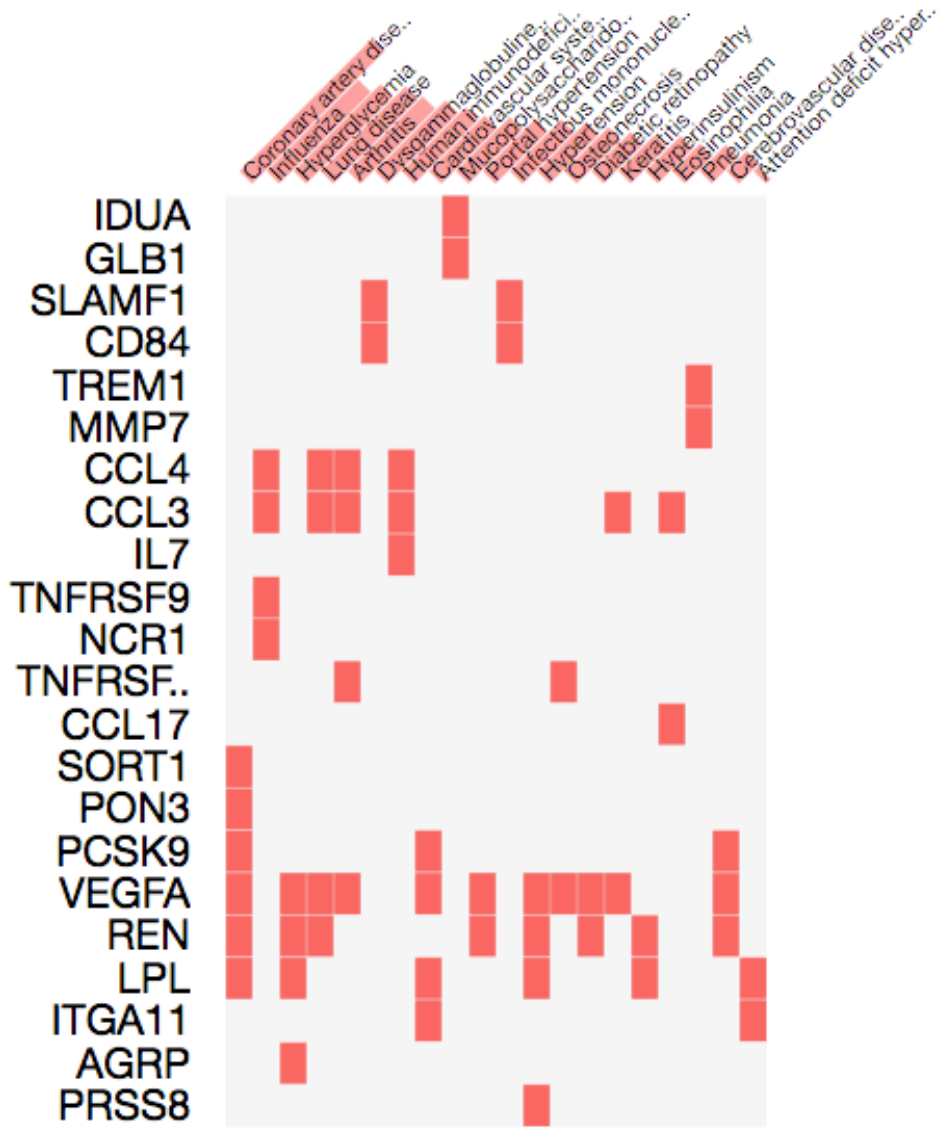
Figure 5.3 Tissue-specific expression of proteins highly expressed in the DIASTRAT cohort

Tissue types expressing proteins highly expressed in the DIASTRAT cohort are ranked based on outputs from the JENSENS TISSUE database. Data are depicted as a heatmap (top panel) and ranked according to significance (lower panel).

5.3.4 The protein signature of the DIASTRAT cohort is consistent with Coronary Artery Disease.

The association of the 75 protein signature with disease phenotypes was assessed. Following imputation of the gene list into Enrichr, the association with disease phenotypes was assessed using the JENSEN DISEASES database (Chen et al., 2013, Kuleshov et al., 2016). This database integrates evidence on disease-gene associations from automatic text mining, manually curated literature, cancer mutation data, and genome-wide association studies. The evidence is further validated by assigning confidence scores that facilitate comparison of the different types and sources of evidence.

The protein signature of the DIASTRAT cohort was most significantly associated with Coronary Artery Disease (CAD; P=0.01) consistent with the findings that CAD is a major complication of T2DM. Interestingly, the protein signature was also significantly associated with a host of other inflammatory conditions including lung disease and arthritis (Figure 5.4). Hyperglycaemia was ranked 3rd in this analysis (P=0.01) consistent with the reported HbA1c values for the cohort. Diabetic retinopathy was ranked 14th in this analysis (P=0.1) and with T2DM ranked 52nd (P=0.3). This may reflect (1) the lack of a defined protein signature for T2DM or (2) the fact that secondary complications of hyperglycaemia are driving the protein signature in this cohort.



Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	Coronary_artery_disease	0.0001118	0.01655	-4.33	39.41
2	Influenza	0.0004984	0.02152	-3.58	27.23
3	Hyperglycemia	0.0006920	0.02152	-3.54	25.76
4	Lung_disease	0.0009944	0.02169	-3.65	25.25
5	Arthritis	0.005022	0.06757	-4.20	22.23
6	Dysgammaglobulinemia	0.0002802	0.02074	-2.57	21.00
7	Human_immunodeficiency_virus_infectious_disease	0.002138	0.03956	-3.09	19.00
8	Cardiovascular_system_disease	0.01313	0.1292	-3.83	16.58
9	Mucopolysaccharidosis	0.0007269	0.02152	-2.22	16.01
10	Portal_hypertension	0.002458	0.04042	-2.57	15.43

Figure 5.4 Disease phenotypes associated with proteins highly expressed in the DIASTRAT cohort

Diseases are ranked based on data obtained from the Jensen lab DISEASES database. Data are depicted as a heatmap (top panel) and ranked according to significance (lower panel).

5.3.5 Effect of prescribing practices on protein expression

Based on rankings outlined in Table 5.1, the 20 proteins with most significant differential expression between control subjects and those in the DIASTRAT cohort were graphed according to prescription of exogenous insulin (INS) or sulphonylurea (SU). As with Chapter 4, this resulted in 5 subgroupings: (1) All participants; (2) SU+/INS+; (3) SU-/INS+; (4) SU+/INS-; and (5) SU-/INS-. All 20 proteins were associated with the immune response in some capacity, consistent with observations outlined in Figure 5.1 – 5.4. Significant differences in protein expression between the 5 subgroupings are discussed below according to the dominant function of the proteins.

Relative protein expression of Gal-4, CD84, Il4-RA, CCL4 and CLEC4A (seen in Figure 5.5), which are known to have effects on cell adhesion and cellular communication. There were no significant differences in the expression of CD84 between any of the subgroupings (Figure 5.5B). In all other instances, the expression of the protein differed significantly (P<0.05-0.0001) between the entire cohort values (All participants) and subgroupings of those on SU and/or INS (Figure 5.5A, C-E). This however, may potentially be explained by the differences in group size and the spread observed throughout the entire cohort versus the smaller groups of participants receiving SU and/or INS. Importantly, SU or INS prescription alone or in combination, did not appear to significantly influence the expression of any of these markers (Figure 5.5).

Chapter 6:

Genetic variants associated with a T2DM phenotype and glycaemic control in the DIASTRAT cohort

6.1 Introduction

Genome-wide association studies (GWAS), linkage analysis, candidate gene approaches and sequencing have allowed for the identification of common, low-frequency and rare variants that are characteristic of T2DM (Stancakova and Laakso, 2016, Fuchsberger et al., 2016). As a result over 80 variants for T2DM have been identified. These variants are common, but yield a small effect with a minor allele frequency >5%, and therefore account only for around 10 % of heritability of T2DM (Stancakova and Laakso, 2016, Fuchsberger et al., 2016), the focus of most studies does tend to be on common variation with larger gene effects. This is a result of the fact that studies have greater power to detect these size effects. Whilst the minor allele frequency does impact the size of gene effect it is not the only factor. How much the variant disrupts the protein for example, also plays a role. The cause of the remaining heritability is thought to be due to the large effects of low frequency and rare variants, interactions between genes, genes and environmental factors or even epigenetic factors (Stancakova and Laakso, 2016, Fuchsberger et al., 2016).

The current study is interested in identifying genetic driver of glycaemic control, particularly in those treated with sulphonylurea (SU). Prior work has unsurprisingly shown that members of the K_{ATP} channel, namely ATP binding site cassette, subfamily C, member 8 (*ABCC8*) that encodes for the sulphonylurea receptor 1 (SUR1) in pancreatic β-cells (Kapoor, 2010, Haghverdizadeh et al., 2014) and the inwardly rectifying potassium channel, subfamily J, member 11 (*KCNJ11*) gene (Qin et al., 2013)., which encodes for Kir6.2, play an essential role in response to SU (Klen et al., 2014). Variants identified in each of these genes that have been associated with an increased risk of T2DM. With *ABCC8* those include S1369A polymorphism (Hansen et al., 1998, Fatehi et al., 2012), which also impacts the efficacy of the SU gliclazide (Feng et al., 2008), R1273R

polymorphism (Rissanen et al., 2000), the -3c → t (rs1799854) (Hart et al., 1999). Some of the variants in KCNJ11 that are associated with a risk of T2DM include rs5219 (Abdelhamid et al., 2014), which can impact the insulin secretion pathway also (Haghvirdizadeh et al., 2015), rs5215 (Chavali et al., 2011) and rs5210 (Koo et al., 2007) which may also improve the efficacy of gliclazide (Haghvirdizadeh et al., 2015). The E23K polymorphism has been widely studied in this gene and shown in some populations to be linked to risk of T2DM (Hani et al., 1998, van Dam et al., 2005, Florez et al., 2007, Abdelhamid et al., 2014).

Variants in hepatocyte nuclear factor-1α (*HNF-1 α*) transcription factor (Hansen et al., 1997, McDonald et al., 2012) have also been implicated in causing increased risk to T2DM and affecting insulin secretion, examples include I27L and A98V (rs1920792) polymorphisms (Holmkvist et al., 2006). Lastly, the potassium voltage-gated channel, KQT-like subfamily, member 1 (*KCNQ1*) gene encodes for the pore-forming subunit of a voltage gated potassium channel (KvLQT1) of the pancreatic β-cell (Schroner et al., 2011, Travers et al., 2013). Prior work has shown that not only are common variants of this gene associated with risk of T2DM, but also impaired insulin secretion and response to SU (Unoki et al., 2008, Schroner et al., 2011, van Vliet-Ostaptchouk et al., 2012). Examples include rs163184 (T>G) polymorphism(Schroner et al., 2011), rs2283228 and rs2237895 (Unoki et al., 2008).

In Chapter 3, the mechanistic impact of altering expression of these genes on SU response was explored. However understanding more about the single nucleotide polymorphisms (SNPs) in these genes in the DIASTRAT cohort is potentially key to understanding more about the response to treatment among this cohort. To this end the aims of this analysis were two fold. First was to determine the prevalence of SNPs associated with genes of interest within the DIASTRAT cohort. Second was to determine their association with glycaemic control in those prescribed

SU

6.2 Materials and Methods

6.2.1 Chemicals

Reagents of analytical grade and deionized water were used. All other chemicals used are listed in Chapter 2, Section 2.1.

6.2.2 Participant Recruitment

Participants were recruited onto the Stratified Medicine, optimizing treatment for Diabetes (DIASTRAT) study as described in Chapter 2, Section 2.2.15.

6.2.3 Processing Blood samples

Blood samples taken from consented participants was processed using the method described in Chapter 2, Section 2.2.16.

6.2.4 DNA Extraction and quantification

DNA extraction from whole blood was performed as described in Chapter 2, Section 2.2.16, and quantified using Qubit Fluorometric Quantitation (ThermoFisher Scientific Inc.). The Qubit allows for quantitation of dsDNA using fluorescence-based quantitation assays.

6.2.5 SNP genotyping using the Affymetrix UK Biobank array

SNP genotyping was performed by Cambridge Genomics Services (Cambridge, UK), for analysis on the Affymetrix UK Biobank array. Details of sample preparation for this array are found in Chapter 2, Section 2.2.21

6.2.6 Variant Calling

In bound SNPs were called using Axiom's Genotype Console software

(Affymetrix, UK) as described in Chapter 2, Section 2.2.21. Samples failing QC (SNP call rate below 97.2% or QC<0.82) were excluded from further analysis.

6.2.7 Statistics

Differences between genotypes were calculated using a two-sample *t*-test for proportions following conversion of percentages to proportions (where 100% = 1). Significance was accepted if $P<0.05$.

6.3 Results

6.3.1 Identifying prevalent variants within the DIASTRAT cohort

This included SNPs associated with ABCC8, KCNJ11, KCNQ1, HNF1α and TCF7L2. Due to time constraints, and thus in an attempt to finish this project DNA was isolated from only 254 DIASTRAT participants and 19 age and gender-matched non-diabetic control participants, and sequenced on the UK Biobank Array (Affymetrix, UK) which contains ~850k unique targets. Variant calling identified 31 unique SNPs associated with the ABCC8 gene, 101 SNPs associated with KCNJ11, 66 SNPs associated with KCNQ1, 38 SNPs with HNF1α and 44 SNPs associated with TCF7L2. SNP attributes are summarized in Tables 6.1 – 6.5 below, which detail the call rate, Minor Allele Frequency (MAF) score and Hardy-Weinberg *P* value. MAF scores revealed that the vast majority of identified SNPs were common variants (MAF>0.05). A significant difference in the call rate between control and DIASTRAT populations was not observed in any instance (Tables 6.1 – 6.5).

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT
rs10961940	A	G	100.00	98.81	0.1842	0.1314	0.5876	0.4884
rs17743658	A	G	100.00	99.70	0.2895	0.2529	0.6491	0.4587
rs3738591	C	G	100.00	100.00	0.0263	0.0448	0.9062	0.6029
rs4741478	T	G	100.00	100.00	0.0263	0.0492	0.9062	0.1784
rs649224	A	G	100.00	100.00	0.0789	0.0807	0.7087	0.7827
rs9458011	T	C	100.00	100.00	0.0263	0.0656	0.9062	0.4519
rs8098346	T	G	100.00	100.00	0.3421	0.4568	0.2123	0.2539
rs75780075	A	G	100.00	100.00	0.0263	0.0194	0.9062	0.8427
rs73017157	A	G	100.00	100.00	0.0000	0.0164	1.0000	0.8560
rs11647027	A	C	100.00	99.70	0.1053	0.0630	0.0545	0.3734
rs59409558	A	C	100.00	98.21	0.0000	0.0106	1.0000	0.9086
rs11942387	A	G	100.00	99.70	0.3947	0.3819	0.3182	0.1391
rs62295555	T	C	100.00	100.00	0.1053	0.1000	0.6081	0.5250
rs12548842	T	C	100.00	100.00	0.2895	0.3745	0.1163	0.4845
rs79345026	T	C	100.00	99.70	0.1842	0.1451	0.3250	0.6131
rs10961999	T	C	100.00	100.00	0.1579	0.1687	0.4138	0.3365
rs111736238	T	G	100.00	100.00	0.0263	0.0269	0.9062	0.7566
rs114570814	A	G	100.00	98.21	0.0263	0.0154	0.9062	0.8514
rs116734533	T	C	100.00	93.42	0.0000	0.0232	1.0000	0.7821
rs78306404	T	C	100.00	99.41	0.0263	0.0015	0.9062	0.9845
rs111577081	T	C	100.00	99.40	0.0526	0.0692	0.8087	0.4169
rs117279260	T	C	100.00	100.00	0.0000	0.0388	1.0000	0.6775
rs117407497	A	G	100.00	99.70	0.0263	0.0286	0.9062	0.7215
rs2666826	T	C	100.00	100.00	0.2105	0.2687	0.2451	0.5581
rs2297775	T	C	100.00	100.00	0.1842	0.2882	0.3250	0.5711
rs10153859	A	G	100.00	99.70	0.0000	0.0270	1.0000	0.4236
rs17597643	A	G	100.00	99.11	0.0263	0.0331	0.9062	0.6963
rs58584712	A	G	100.00	100.00	0.2368	0.2000	0.1761	0.3753
rs78504459	T	C	100.00	99.70	0.0526	0.0240	0.8087	0.7868
rs150028933	-	A	100.00	100.00	0.0000	0.0194	1.0000	0.4222
rs200349598	A	C	100.00	100.00	0.0000	0.0015	1.0000	0.9845

Table 6.1 Attributes of SNPs associated with ABCC8

31 unique SNPs associated with ABCC8 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* Value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT
rs1030986	T	C	100	100	0.0000	0.0075	1.0000	0.9315
rs16875779	A	G	100	100	0.1316	0.1747	0.5090	0.4333
rs17062197	A	G	100	100	0.2105	0.1254	0.2451	0.3358
rs2517646	T	C	100	99.7	0.3421	0.2977	0.8196	0.2734
rs2607614	T	C	100	100	0.0000	0.0254	1.0000	0.7718
rs33949518	T	C	100	99.405	0.1316	0.1742	0.5090	0.4336
rs34505188	A	G	100	99.4	0.0526	0.0678	0.8087	0.4241
rs35978505	T	C	100	100	0.0789	0.0239	0.7087	0.7871
rs369738	T	G	100	98.8	0.0000	0.0545	1.0000	0.3253
rs3815082	A	G	100	98.51	0.2895	0.2559	0.5090	0.8515
rs2849089	T	C	100	100	0.0000	0.0000	1.0000	1.0000
rs4479449	A	G	100	100	0.0526	0.0940	0.0000	0.2627
rs454748	A	G	100	99.7	0.5000	0.3924	0.4913	0.2435
rs683687	A	G	100	100	0.0526	0.0761	0.8087	0.6767
rs683866	T	G	100	99.7	0.2368	0.4099	0.9335	0.5936
rs6919617	A	G	100	100	0.1053	0.0821	0.6081	0.5434
rs7431770	A	G	100	99.105	0.0000	0.0828	1.0000	0.6365
rs757260	T	C	100	100	0.1316	0.1358	0.5090	0.5447
rs920829	T	C	100	99.4	0.1053	0.1182	0.6081	0.5161
rs9261151	A	G	100	100	0.0526	0.0358	0.8087	0.6752
rs9261216	A	G	100	99.7	0.1053	0.0809	0.6081	0.5118
rs9261302	A	G	100	100	0.1053	0.0821	0.6081	0.5434
rs9261471	T	C	100	100	0.2895	0.2329	0.5090	0.7592
rs9268135	A	G	100	99.405	0.2895	0.2553	0.5090	0.3965
rs9366752	T	C	100	100	0.1842	0.1792	0.5876	0.7184
rs9811423	C	G	100	100	0.3947	0.4030	0.9698	0.3430
rs1693703	T	C	100	98.805	0.1579	0.2781	0.3638	0.6514
rs7841649	T	C	100	98.81	0.0789	0.0121	0.7087	0.8933

Table 6.2 Attributes of SNPs associated with KCNJ11

101 unique SNPs associated with KCNJ11 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT
rs76592289	A	G	100	100	0.0000	0.0463	1.0000	0.4392
rs76557252	T	G	100	100	0.0789	0.0045	0.7087	0.9626
rs506757	T	G	100	99.7	0.2632	0.3232	0.7087	0.3082
rs62166939	A	C	100	97.915	0.1842	0.2115	0.5876	0.4693
rs78082945	T	C	100	100	0.0263	0.0328	0.9062	0.7121
rs62272114	A	G	100	99.7	0.0000	0.0255	1.0000	0.7714
rs112288451	T	C	100	97.905	0.0000	0.0153	1.0000	0.8605
rs74806059	T	C	100	100	0.0000	0.0179	1.0000	0.8316
rs79876945	A	G	100	100	0.0526	0.0299	0.8087	0.7343
rs114687906	A	C	94.74	99.105	0.0000	0.0196	1.0000	0.8241
rs115477611	T	C	100	100	0.0526	0.0180	0.8087	0.8150
rs78771971	T	C	100	100	0.0000	0.0030	1.0000	0.9781
rs78854530	A	G	100	100	0.0000	0.0090	1.0000	0.9159
rs62503295	T	C	94.74	100	0.0556	0.0731	0.8029	0.6219
rs114756881	T	C	94.74	94.62	0.0000	0.0475	1.0000	0.5907
rs4498896	T	C	100	100	0.3947	0.3701	0.3182	0.2276
rs79035182	T	G	100	100	0.0000	0.0194	1.0000	0.8249
rs74898314	A	G	100	100	0.0000	0.0209	1.0000	0.4352
rs28445507	A	C	100	99.7	0.2895	0.2291	0.5090	0.6970
rs55851518	A	G	100	100	0.0789	0.0537	0.7087	0.4286
rs79263726	A	C	100	100	0.0000	0.0224	1.0000	0.8026
rs10898365	T	G	100	100	0.2105	0.2672	0.8275	0.4778
rs66546253	A	G	100	99.4	0.3158	0.2451	0.0254	0.3526
rs783307	T	C	100	100	0.0789	0.1553	0.7087	0.7106
rs17734780	T	C	100	100	0.0000	0.0120	1.0000	0.8847
rs10432095	A	C	100	100	0.1316	0.1522	0.5090	0.5252
rs75107770	A	G	100	100	0.0263	0.0224	0.9062	0.8026
rs13061963	T	G	100	100	0.1053	0.0537	0.0545	0.5286
rs60400172	T	G	100	100	0.0526	0.0671	0.0000	0.0000
rs76451747	A	C	100	100	0.0000	0.0060	1.0000	0.9378
rs12520495	A	G	100	100	0.3421	0.3105	0.4288	0.4288

Table 6.2 Attributes of SNPs associated with KCNJ11 (continued)

101 unique SNPs associated with KCNJ11 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT
rs13183249	A	G	100	100	0.2368	0.2374	0.9335	0.3232
rs72725509	T	C	100	100	0.0000	0.0060	1.0000	0.9471
rs80184435	A	G	100	98.505	0.0263	0.0257	0.9062	0.7786
rs62404592	A	C	100	97.915	0.0000	0.0121	1.0000	0.8840
rs117910348	A	C	100	100	0.0000	0.0134	1.0000	0.8872
rs79853069	A	G	100	99.105	0.1842	0.0858	0.3250	0.6422
rs71521249	A	G	100	100	0.0000	0.0074	1.0000	0.9406
rs114234140	A	G	100	100	0.0263	0.0179	0.9062	0.8231
rs114249268	A	G	100	92.815	0.0000	0.0633	1.0000	0.0023
rs112623189	A	G	100	100	0.1053	0.0388	0.6081	0.6530
rs115010323	T	C	100	99.7	0.0000	0.0105	1.0000	0.9000
rs116250326	T	C	100	99.7	0.0263	0.0045	0.9062	0.9532
rs77111308	T	C	100	100	0.0263	0.0582	0.9062	0.4949
rs77823977	T	C	100	100	0.0000	0.0179	1.0000	0.8404
rs78374474	T	C	100	100	0.0789	0.0179	0.7087	0.4899
rs192657690	T	C	100	100	0.0000	0.0075	1.0000	0.9315
rs116983235	T	C	100	100	0.0000	0.0030	1.0000	0.9781
rs10846754	A	G	100	100	0.1316	0.1956	0.5090	0.6429
rs576184	A	C	100	100	0.3684	0.3851	0.1195	0.8357
rs16951460	A	G	100	97.305	0.0000	0.0327	1.0000	0.4338
rs3748592	A	G	100	100	0.0526	0.0686	0.8087	0.4332
rs2096376	T	C	100	100	0.1842	0.2880	0.3250	0.1887
rs32555	A	G	100	99.4	0.1579	0.1771	0.4138	0.3994
rs9261089	T	C	100	99.7	0.0526	0.0360	0.8087	0.6746
rs28400886	A	G	100	100	0.0263	0.0493	0.9062	0.5988
rs4959042	T	C	100	100	0.0000	0.0060	1.0000	0.9471
rs34704616	A	G	100	100	0.0000	0.0030	1.0000	0.9781
rs1573298	C	G	100	99.405	0.2895	0.2567	0.5090	0.7659
rs397596	T	C	100	100	0.0000	0.0000	1.0000	1.0000
rs139080520	A	G	100	100	0.0000	0.0000	1.0000	1.0000

Table 6.2 Attributes of SNPs associated with KCNJ11 (continued)

101 unique SNPs associated with KCNJ11 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT
rs13268757	A	G	100	100	0.1316	0.1567	0.1779	0.9487
rs114814001	T	C	100	100	0.0000	0.0254	1.0000	0.7718
rs79765628	T	C	100	100	0.0263	0.0254	0.9062	0.7638
rs117389196	T	C	100	99.7	0.0000	0.0194	1.0000	0.8425
rs141834826	A	G	100	99.7	0.0000	0.0000	1.0000	1.0000
rs201447432	T	C	100	99.1	0.0000	0.0137	1.0000	0.8589
rs146548891	T	G	100	99.7	0.0000	0.0030	1.0000	0.9781
rs142720326	A	G	100	100	0.0000	0.0030	1.0000	0.9781
rs201960928	A	G	100	99.7	0.0000	0.0105	1.0000	0.8907
rs139889337	A	G	100	99.7	0.0000	0.0090	1.0000	0.9249
rs113993403	T	C	100	100	0.0000	0.0089	1.0000	0.9250
rs138962514	T	C	100	100	0.0000	0.0000	1.0000	1.0000

Table 6.2 Attributes of SNPs associated with KCNJ11 (final)

101 unique SNPs associated with KCNJ11 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT
rs16841998	T	C	84.21	89.26	0.1250	0.0882	0.5677	0.3247
rs17461843	T	C	100	97.32	0.0000	0.0460	1.0000	0.6039
rs17571372	T	C	100	100.00	0.0263	0.0538	0.9062	0.1909
	T	G	100	99.70	0.0000	0.0000	1.0000	1.0000
rs3006968	A	G	100	99.70	0.5000	0.4730	0.8185	0.3712
rs6039591	A	G	100	100.00	0.0000	0.0239	1.0000	0.7871
rs6883010	A	G	100	100.00	0.3158	0.4164	0.3421	0.4232
rs6133707	A	G	100	99.70	0.2368	0.2185	0.1761	0.8125
rs77707280	A	G	100	99.70	0.0526	0.0568	0.8087	0.4589
rs807936	T	C	100	100.00	0.3158	0.3984	0.3421	0.4444
rs114348392	T	G	94.74	99.70	0.0278	0.0150	0.9035	0.8714
rs9356928	A	G	100	99.40	0.4211	0.4653	0.7288	0.6553
rs9460973	A	T	100	99.70	0.0000	0.0449	1.0000	0.6087
rs58554303	T	C	100	99.70	0.0263	0.0269	0.9062	0.7645
rs920628	A	G	100	100.00	0.0263	0.0388	0.9062	0.3248
rs16873732	T	C	100	100.00	0.0263	0.0388	0.9062	0.3248
rs1041791	T	C	100	99.70	0.2105	0.1870	0.2451	0.5258
rs61741363	A	G	100	100.00	0.0000	0.0000	1.0000	1.0000
rs113319303	A	G	100	99.70	0.0000	0.0435	1.0000	0.3893
rs116581086	A	G	100	100.00	0.0000	0.0045	1.0000	0.9626
rs74745471	A	G	100	100.00	0.0263	0.0567	0.9062	0.5118
rs61936141	T	C	100	99.70	0.0000	0.0180	1.0000	0.8312
rs79090092	A	G	100	100.00	0.0263	0.0389	0.9062	0.6288
rs10905750	A	C	100	100.00	0.0000	0.0239	1.0000	0.7871
rs231847	A	G	100	100.00	0.3158	0.4075	0.3421	0.0792
rs129072	T	C	100	99.70	0.0789	0.1737	0.7087	0.6597
rs75898263	T	C	100	100.00	0.0000	0.0000	1.0000	1.0000
rs111871569	A	G	100	99.41	0.0526	0.0376	0.8087	0.3675

Table 6.3 Attributes of SNPs associated with KCNQ1

68 unique SNPs associated with KCNQ1 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT
rs75875515	A	G	100	99.40	0.0000	0.0211	1.0000	0.8001
rs7156987	T	C	100	98.22	0.0000	0.0183	1.0000	0.8476
rs55767542	T	C	100	99.40	0.0000	0.0210	1.0000	0.8086
rs35162844	A	G	100	99.70	0.0789	0.0389	0.7087	0.3243
rs73063633	T	G	100	99.70	0.0263	0.0240	0.9062	0.7868
rs116045044	T	G	100	99.11	0.0263	0.0407	0.9062	0.6517
rs77692939	A	G	100	97.91	0.0000	0.0076	1.0000	0.9306
rs72811968	T	C	100	100.00	0.0000	0.0164	1.0000	0.8649
rs76238538	T	C	100	100.00	0.0000	0.0269	1.0000	0.7649
rs79931282	A	G	100	99.41	0.0526	0.0330	0.8087	0.7113
rs9467059	A	G	100	99.11	0.0000	0.0512	1.0000	0.5524
rs188539852	T	C	100	100.00	0.0263	0.0119	0.9062	0.9028
rs115704940	T	C	100	100.00	0.0263	0.0299	0.9062	0.4514
rs116858972	T	C	100	100.00	0.0263	0.0194	0.9062	0.8249
rs117103115	A	G	100	96.12	0.0000	0.0311	1.0000	0.7210
rs77936291	A	G	100	100.00	0.0263	0.0284	0.9062	0.7277
rs79947987	T	C	100	99.70	0.0000	0.0030	1.0000	0.9781
rs78468689	A	G	100	100.00	0.0263	0.0373	0.9062	0.6675
rs80140016	T	G	100	99.70	0.0526	0.0389	0.8087	0.6524
rs75894327	A	G	100	100.00	0.0000	0.0358	1.0000	0.6901
rs114351051	T	C	100	94.65	0.0000	0.0627	1.0000	0.4832
rs115005266	T	C	100	99.41	0.0263	0.0225	0.9062	0.8020
rs112214748	A	G	100	100.00	0.1316	0.0269	0.5090	0.7489
rs7119884	A	G	100	100.00	0.0263	0.0343	0.9062	0.7144
rs6057010	A	G	100	100.00	0.0526	0.0642	0.8087	0.4512
rs4690087	A	G	100	100.00	0.1316	0.0732	0.5090	0.3671
rs11754464	T	C	100	99.70	0.0000	0.0000	1.0000	1.0000
rs707938	A	G	100	99.41	0.3421	0.3468	0.8196	0.1125

Table 6.3 Attributes of SNPs associated with KCNQ1 (continued)

68 unique SNPs associated with KCNQ1 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT
rs35185125	A	C	100	100.00	0.0263	0.0060	0.9062	0.9471
rs28381355	A	G	100	100.00	0.0000	0.0060	1.0000	0.9378
rs183314661	A	C	100	100.00	0.0000	0.0239	1.0000	0.7788
rs77320475	A	G	100	99.70	0.0789	0.0359	0.7087	0.6816
rs17812699	A	G	100	100.00	0.0000	0.0791	1.0000	0.6406
rs199853687	A	C	100	100.00	0.0000	0.0000	1.0000	1.0000
rs115491500	T	C	100	100.00	0.0000	0.0000	1.0000	1.0000
rs11553182	T	C	100	100.00	0.0789	0.0895	0.7087	0.2887
	C	G	100	100.00	0.0000	0.0015	1.0000	0.9845
rs341047	A	G	100	99.41	0.1579	0.1080	0.4138	0.4724
rs150934987	A	G	100	100.00	0.0000	0.0000	1.0000	1.0000
rs139577182	A	G	100	100.00	0.0000	0.0045	1.0000	0.9626

Table 6.3 Attributes of SNPs associated with KCNQ1 (final)

68 unique SNPs associated with KCNQ1 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT
rs11755881	A	G	100.00	100.00	0.0263	0.1000	0.9062	0.5564
rs1861	A	C	100.00	100.00	0.0263	0.0254	0.9062	0.3895
rs28568667	T	C	89.47	97.01	0.1471	0.1237	0.4772	0.1781
rs4770748	A	G	100.00	100.00	0.0000	0.0702	1.0000	0.7022
rs663824	A	G	100.00	100.00	0.4211	0.3567	0.5522	0.6183
rs6905159	A	G	100.00	99.40	0.4474	0.4475	0.4564	0.9362
rs888576	T	C	100.00	100.00	0.1579	0.0880	0.4138	0.1605
rs13325508	T	C	100.00	100.00	0.0263	0.0239	0.9062	0.7959
rs55723035	T	C	100.00	100.00	0.0000	0.0149	1.0000	0.8626
rs77356515	T	G	100.00	100.00	0.0526	0.0090	0.8087	0.9159
rs75363174	A	G	100.00	100.00	0.0526	0.0582	0.8087	0.4421
rs4751674	T	C	100.00	99.70	0.3947	0.3039	0.3182	0.2773
rs61433965	A	G	100.00	99.70	0.0000	0.0150	1.0000	0.8714
rs58838391	A	G	100.00	100.00	0.0000	0.0149	1.0000	0.8626
rs76280424	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs77794667	T	C	100.00	100.00	0.0263	0.0164	0.9062	0.8560
rs59559871	T	C	100.00	99.70	0.0000	0.0000	1.0000	1.0000
rs72826927	A	G	100.00	100.00	0.0526	0.0687	0.8087	0.6969
rs945094	A	T	100.00	100.00	0.0263	0.0254	0.9062	0.7564
rs72751116	A	G	100.00	100.00	0.0000	0.0030	1.0000	0.9690
rs59706474	T	C	100.00	100.00	0.0263	0.0164	0.9062	0.8649
rs73251982	A	G	100.00	98.21	0.0789	0.0291	0.7087	0.4338
rs72489176	A	G	100.00	100.00	0.2368	0.2134	0.9335	0.2682
rs77367389	A	C	100.00	100.00	0.0263	0.0074	0.9062	0.9406
rs73668591	T	C	100.00	99.70	0.0000	0.0090	1.0000	0.9249
rs73669423	T	C	100.00	100.00	0.0789	0.0761	0.7087	0.3774
rs62493228	A	C	100.00	100.00	0.0789	0.0522	0.7087	0.5461
rs76569571	T	G	100.00	98.22	0.0000	0.0229	1.0000	0.8094

Table 6.4 Attributes of SNPs associated with HNF1α

38 unique SNPs associated with HNF1α were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT
rs80312260	A	G	100.00	100.00	0.0526	0.0179	0.8087	0.8404
rs2244608	A	G	100.00	100.00	0.3158	0.2806	0.0442	0.3364
rs11616995	A	G	100.00	100.00	0.0000	0.0164	1.0000	0.8560
rs11647932	T	C	100.00	99.70	0.2105	0.1079	0.2451	0.4511
rs8102561	T	C	100.00	100.00	0.2105	0.1627	0.8275	0.6045
rs340141	T	C	100.00	99.70	0.3158	0.3306	0.9110	0.6290
rs150333766	T	C	100.00	100.00	0.0000	0.0045	1.0000	0.9626
rs189023122	T	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs150428096	T	C	100.00	99.10	0.0000	0.0061	1.0000	0.9372
rs147799118	T	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000

Table 6.4 Attributes of SNPs associated with HNF1α (final)

38 unique SNPs associated with HNF1α were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT
rs12894204	A	G	100.00	100.00	0.1842	0.2015	0.3250	0.1198
rs17605420	A	G	100.00	100.00	0.0263	0.0194	0.9062	0.8163
rs17763253	T	C	100.00	100.00	0.0526	0.0492	0.8087	0.2809
rs6728493	A	C	100.00	100.00	0.3158	0.2761	0.9110	0.3339
rs9906935	A	G	100.00	99.70	0.3947	0.2949	0.3564	0.5542
rs7089262	T	C	100.00	99.70	0.0000	0.0389	1.0000	0.6679
rs76677854	T	C	100.00	99.70	0.0789	0.0434	0.7087	0.6162
rs145079521	A	C	100.00	99.70	0.0526	0.0330	0.8087	0.7038
rs78082945	T	C	100.00	100.00	0.0263	0.0328	0.9062	0.7121
rs62272114	A	G	100.00	99.70	0.0000	0.0255	1.0000	0.7714
rs77385406	A	G	100.00	100.00	0.1053	0.0298	0.6081	0.7427
rs61732118	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs61875109	A	C	100.00	99.70	0.4211	0.2201	0.5522	0.6466
rs116369954	T	C	100.00	99.40	0.0000	0.0465	1.0000	0.3649
rs79805154	A	G	100.00	99.70	0.0526	0.0704	0.8087	0.7404
rs77064952	A	G	100.00	99.41	0.0000	0.0075	1.0000	0.9314
rs10059523	A	G	100.00	100.00	0.0263	0.0298	0.9062	0.7517
rs78183526	T	C	100.00	100.00	0.0000	0.0015	1.0000	0.9845
rs587667473	A	C	100.00	99.70	0.2632	0.1825	0.1195	0.0162
rs73179006	T	G	100.00	100.00	0.0526	0.0388	0.8087	0.6684
rs113262704	T	G	100.00	99.70	0.0526	0.0298	0.8087	0.7809
rs61989114	A	G	100.00	100.00	0.0000	0.0075	1.0000	0.9315
rs55656505	T	C	100.00	100.00	0.0000	0.0433	1.0000	0.5489
rs4783943	A	G	100.00	100.00	0.2368	0.2491	0.1761	0.5079
rs3026084	A	G	100.00	98.81	0.0000	0.0242	1.0000	0.7945
rs189314829	A	G	100.00	99.40	0.0000	0.0270	1.0000	0.7641
rs75107770	A	G	100.00	100.00	0.0263	0.0224	0.9062	0.8026
rs73223028	T	C	100.00	100.00	0.0526	0.0119	0.8087	0.8938
rs116929578	A	G	100.00	99.41	0.0263	0.0480	0.9062	0.6570

Table 6.5 Attributes of SNPs associated with TCF7L2

44 unique SNPs associated with TCF7L2 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT
rs117041486	A	G	100.00	100.00	0.0526	0.0104	0.8087	0.9094
rs79565172	A	G	100.00	99.41	0.0263	0.0090	0.9062	0.9247
rs117582734	T	C	100.00	100.00	0.0789	0.0552	0.7087	0.5163
rs12243326	T	C	100.00	100.00	0.3158	0.3582	0.9110	0.6068
rs4889830	A	G	100.00	100.00	0.4737	0.4791	0.4977	0.6780
rs3787493	A	G	100.00	99.70	0.4474	0.4684	0.8547	0.3243
rs4713505	T	G	100.00	100.00	0.3684	0.2015	0.0110	0.6215
rs2070600	T	C	100.00	99.70	0.1842	0.0689	0.3250	0.4181
rs3131300	A	G	100.00	99.70	0.2632	0.2559	0.7087	0.1733
rs73471190	T	C	100.00	99.41	0.0000	0.0030	1.0000	0.9780
rs148924158	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs143828311	T	C	100.00	100.00	0.0263	0.0104	0.9062	0.9094
rs201967398	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs143652701	T	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs142236510	T	C	100.00	100.00	0.0000	0.0239	1.0000	0.7959

Table 6.5 Attributes of SNPs associated with TCF7L2 (final)

44 unique SNPs associated with TCF7L2 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

6.3.2 Variation in genotype between DIASTRAT and control cohorts.

Next, genotyping was performed using the Axiom Genotype Console programme. Overall, the genotypes of control and DIASTRAT cohorts were largely comparable as shown in Tables 6.6 – 6.10. No significant difference in the prevalence of any SNP associated with ABCC8 was observed between the control and DIASTRAT cohorts (Table 6.6). Consistently, differences were not observed in the prevalence of SNPs associated with KCNQ1 (Table 6.8) and HNF1α (Table 6.9). However, there were several exceptions, which are summarized in Table 6.11.

KCNJ11

As is shown below in Table 6.7, the AB genotype of rs7841649 was more commonly observed in the non-diabetic control cohort compared to the diabetic cohort (15.79% control versus 2.39% DIASTRAT, *P*=0.01, 95% CI -0.06, 0.33 OR 7.6) . This is similar to the AB genotype of rs78374474 (15.79% control versus 2.99% DIASTRAT, *P*=0.03, 95% CI -0.07, 0.32, OR 6.08). Conversely, the BB genotype of this SNP was more common among the diabetic cohort (84.21% control versus 96.71% DIASTRAT, *P*=0.04, 95% CI -0.32, 0.067, OR 0.181). The AB genotype of rs76557252 was also more dominant in the non-diabetic control cohort (15.79% control versus 0.89% DIASTRAT, *P*=0.0002, 95% CI -0.04, 0.34, OR 20.77), whilst the BB genotype for this SNP was more commonly observed in the diabetic cohort (84.21% control versus 99.11% DIASTRAT, *P*=0.0002, 95% CI -0.343, 0.043, OR 0.048). Clustering of genotypes between the control and DIASTRAT cohorts are shown graphically for rs78374474 and rs76557252 in Figure 6.1 and 6.2 respectively. Despite significant *P* values and ORs the instability of the CIs mean that these SNPs are unlikely to have significant pathogenic effect. Furthermore, data from the T2D Knowledge portal does not identify any of these variants as being significantly associated with a T2DM phenotype.

TCF7L2

As is shown in Table 6.10, the AB genotype of rs4713505 was more commonly observed in the non-diabetic control cohort than in the diabetic cohort (73.68% control versus 30.75% DIASTRAT, *P*=0.0004, 95% CI 0.196, 0.664, OR 6.3); however, the opposite was seen in the BB genotype of the same SNP (26.32% control versus 64.48% DIASTRAT, *P*=0.002, 95% CI -0.617, -0.148, OR 0.197). Clustering of these genotypes is presented graphically in Figure 6.3. This SNP is associated with a wide range of phenotypes including, T2DM, height, waist-hip ratio, fasting insulin, coronary artery disease, HDL cholesterol and fasting glucose, according to data presented on the T2D Knowledge portal.

			% AA						% AB						% BB					
dbSNP RS ID	Minor allele (A)	Major allele (B)	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio
rs10961940	A	G	68.42	74.33	0.780	-0.304	0.184	0.748	26.32	22.99	0.962	-0.198	0.268	1.197	5.26	2.38	0.992	-0.101	0.159	2.278
rs17743658	A	G	10.53	7.46	0.973	-0.140	0.200	1.460	36.84	35.53	1.000	-0.225	0.253	1.059	52.63	57.14	0.887	-0.305	0.216	0.833
rs3738591	C	G	94.74	91.04	0.874	-0.097	0.173	1.771	5.26	8.96	0.872	-0.173	0.097	0.565	0.00	0.00	1.000	0.000	0.000	0.000
rs4741478	T	G	94.74	91.06	0.877	-0.097	0.173	1.768	5.26	8.05	0.874	-0.158	0.106	0.635	0.00	1.19	1.000	-0.037	0.013	0.000
rs649224	A	G	0.00	0.60	1.000	-0.027	0.011	0.000	15.79	14.94	1.000	-0.170	0.186	1.068	84.21	89.29	0.793	-0.248	0.145	0.640
rs9458011	T	C	0.00	0.00	1.000	0.000	0.000	0.000	5.26	13.13	0.294	-0.214	0.060	0.368	94.74	84.52	0.187	-0.037	0.239	3.296
rs8098346	T	G	5.26	19.70	0.210	-0.284	-0.004	0.226	57.89	51.96	0.792	-0.199	0.318	1.271	36.84	34.52	1.000	-0.225	0.269	1.106
rs75780075	A	G	94.74	96.13	1.000	-0.130	0.103	0.725	5.26	3.87	1.000	-0.103	0.130	1.378	0.00	0.00	1.000	0.000	0.000	0.000
rs73017157	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	3.28	1.000	-0.081	0.018	0.000	100.00	96.43	0.524	-0.016	0.086	0.000
rs11647027	A	C	84.21	88.34	0.880	-0.237	0.157	0.704	10.53	10.16	1.000	-0.143	0.149	1.040	5.26	0.00	0.635	-0.076	0.181	0.000
rs59409558	A	C	100.00	96.12	0.385	-0.013	0.092	0.000	0.00	2.09	1.000	-0.056	0.017	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs11942387	A	G	21.05	12.85	0.575	-0.136	0.297	1.808	36.84	50.43	0.349	-0.389	0.118	0.573	42.11	35.71	0.761	-0.195	0.321	1.309
rs62295555	T	C	0.00	0.60	1.000	-0.027	0.011	0.000	21.05	18.80	1.000	-0.190	0.233	1.152	78.95	79.76	1.000	-0.209	0.190	0.951
rs12548842	T	C	15.79	15.81	1.000	-0.170	0.171	0.998	26.32	43.27	0.182	-0.405	0.066	0.468	57.89	33.33	0.063	-0.013	0.502	2.750
rs79345026	T	C	0.00	1.49	1.000	-0.047	0.015	0.000	36.84	25.96	0.480	-0.143	0.360	1.664	63.16	69.05	0.789	-0.310	0.195	0.768
rs10961999	T	C	0.00	4.17	0.285	-0.097	0.010	0.000	31.58	25.38	0.759	-0.180	0.308	1.357	68.42	72.62	0.901	-0.285	0.204	0.817
rs111736238	T	G	94.74	94.63	1.000	-0.104	0.109	1.022	5.26	5.37	1.000	-0.109	0.104	0.978	0.00	0.00	1.000	0.000	0.000	0.000
rs114570814	A	G	94.74	95.21	1.000	-0.115	0.104	0.905	5.26	2.99	0.880	-0.103	0.145	1.802	0.00	0.00	1.000	0.000	0.000	0.000
rs116734533	T	C	100.00	89.23	0.266	0.040	0.172	0.000	0.00	4.19	0.279	-0.097	0.010	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs78306404	T	C	0.00	0.00	1.000	0.000	0.000	0.000	5.26	0.30	0.678	-0.080	0.178	18.500	94.74	98.81	0.810	-0.170	0.089	0.217
rs111577081	T	C	0.00	0.00	1.000	0.000	0.000	0.000	10.53	13.74	0.959	-0.205	0.140	0.739	89.47	88.10	1.000	-0.144	0.169	1.149
rs117279260	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	7.75	0.424	-0.140	-0.017	0.000	100.00	89.29	0.269	0.040	0.172	0.000
rs117407497	A	G	94.74	94.02	1.000	-0.105	0.117	1.146	5.26	5.69	1.000	-0.109	0.104	0.922	0.00	0.00	1.000	0.000	0.000	0.000
rs2666826	T	C	57.89	53.13	0.869	-0.211	0.306	1.213	42.11	40.00	1.000	-0.230	0.269	1.091	0.00	4.76	0.148	-0.102	0.007	0.000
rs2297775	T	C	63.16	51.63	0.449	-0.138	0.370	1.606	36.84	39.11	1.000	-0.268	0.225	0.908	0.00	5.95	0.566	-0.116	-0.002	0.000
rs10153859	A	G	0.00	0.30	1.000	-0.016	0.008	0.000	0.00	4.78	0.145	-0.102	0.007	0.000	100.00	95.24	0.148	-0.007	0.102	0.000
rs17597643	A	G	94.74	92.53	1.000	-0.105	0.150	1.453	5.26	6.57	1.000	-0.134	0.105	0.790	0.00	0.00	1.000	0.000	0.000	0.000
rs58584712	A	G	0.00	5.67	0.593	-0.111	0.001	0.000	47.37	28.67	0.179	-0.073	0.446	2.239	52.63	70.24	0.211	-0.434	0.085	0.471
rs78504459	T	C	89.47	94.92	0.715	-0.223	0.115	0.454	10.53	4.78	0.684	-0.111	0.227	2.346	0.00	0.00	1.000	0.000	0.000	0.000
rs150028933	-	A	100.00	96.72	0.686	-0.018	0.081	0.000	0.00	2.69	1.000	-0.075	0.020	0.000	0.00	1.19	1.000	-0.037	0.013	0.000
rs200349598	A	C	100.00	99.70	1.000	-0.008	0.016	0.000	0.00	0.30	1.000	-0.016	0.008	0.000	0.00	0.00	1.000	0.000	0.000	0.000

Table 6.6 SNPs associated with ABCC8

31 unique SNPs associated with ABCC8 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$.

			% AA						% AB						% BB					
dbSNP RS ID	Minor allele (A)	Major allele (B)	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		C
rs1030986	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.49	1.00	-0.05	0.02	0.00	100.00	98.51	1.00	-0.015	0.047	
rs16875779	A	G	0.00	4.48	0.73	-0.10	0.01	0.00	26.32	25.98	1.00	-0.21	0.21	1.02	73.68	69.54	0.90	-0.194	0.274	
rs17062197	A	G	0.00	1.20	1.00	-0.04	0.01	0.00	42.11	22.68	0.10	-0.06	0.45	2.48	57.89	76.12	0.14	-0.437	0.076	
rs2517646	T	C	42.11	51.35	0.59	-0.35	0.17	0.69	47.37	37.31	0.53	-0.16	0.36	1.51	10.53	11.04	1.00	-0.153	0.143	
rs2607614	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	5.08	0.66	-0.11	0.00	0.00	100.00	94.92	0.66	-0.004	0.107	
rs33949518	T	C	0.00	4.18	0.77	-0.10	0.01	0.00	26.32	26.27	1.00	-0.21	0.21	1.00	73.68	68.95	0.86	-0.186	0.282	
rs34505188	A	G	0.00	0.90	1.00	-0.03	0.01	0.00	10.53	11.65	1.00	-0.17	0.14	0.89	89.47	86.85	1.00	-0.144	0.193	
rs35978505	T	C	84.21	95.22	0.13	-0.30	0.08	0.27	15.79	4.78	0.13	-0.08	0.30	3.74	0.00	0.00	1.00	0.000	0.000	
rs369738	T	G	100.00	88.95	0.26	0.04	0.18	0.00	0.00	8.96	0.35	-0.15	-0.03	0.00	0.00	0.89	1.00	-0.027	0.011	
rs3815082	A	G	47.37	54.92	0.69	-0.33	0.19	0.74	47.37	36.73	0.50	-0.15	0.37	1.55	5.26	6.87	1.00	-0.134	0.105	
rs2849089	T	C	100.00	100.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.000	0.000	
rs4479449	A	G	94.74	81.19	0.24	0.00	0.28	4.17	0.00	18.81	0.08	-0.27	-0.11	0.00	5.26	0.00	0.09	-0.076	0.181	
rs454748	A	G	21.05	16.71	0.87	-0.17	0.26	1.33	57.89	44.80	0.39	-0.13	0.39	1.69	21.05	38.19	0.21	-0.392	0.050	
rs683687	A	G	0.00	0.89	1.00	-0.03	0.01	0.00	10.53	13.43	0.99	-0.20	0.14	0.76	89.47	85.68	0.91	-0.136	0.209	
rs683866	T	G	57.89	33.74	0.06	-0.02	0.50	2.70	36.84	50.15	0.38	-0.39	0.12	0.58	5.26	15.81	0.36	-0.243	0.033	
rs6919617	A	G	78.95	84.17	0.78	-0.27	0.16	0.71	21.05	15.23	0.73	-0.16	0.27	1.48	0.00	0.60	1.00	-0.027	0.011	
rs7431770	A	G	0.00	0.60	1.00	-0.03	0.01	0.00	0.00	15.23	0.13	-0.23	-0.08	0.00	100.00	83.28	0.11	0.091	0.239	
rs757260	T	C	0.00	1.19	1.00	-0.04	0.01	0.00	26.32	24.78	1.00	-0.21	0.24	1.08	73.68	74.03	1.00	-0.212	0.205	
rs920829	T	C	0.00	0.30	1.00	-0.02	0.01	0.00	21.05	22.96	1.00	-0.23	0.19	0.89	78.95	76.14	1.00	-0.189	0.249	
rs9261151	A	G	0.00	0.00	1.00	0.00	0.00	0.00	10.53	7.17	0.93	-0.14	0.20	1.52	89.47	92.83	0.93	-0.204	0.135	
rs9261216	A	G	78.95	84.17	0.78	-0.27	0.16	0.71	21.05	14.93	0.70	-0.16	0.28	1.52	0.00	0.60	1.00	-0.027	0.011	
rs9261302	A	G	0.00	0.60	1.00	-0.03	0.01	0.00	21.05	15.23	0.73	-0.16	0.27	1.48	78.95	84.17	0.78	-0.270	0.164	
rs9261471	T	C	47.37	59.09	0.45	-0.38	0.14	0.62	47.37	35.24	0.42	-0.14	0.38	1.65	5.26	5.67	1.00	-0.109	0.104	
rs9268135	A	G	47.37	55.82	0.63	-0.35	0.18	0.71	47.37	36.42	0.48	-0.15	0.37	1.57	5.26	7.16	1.00	-0.142	0.105	
rs9366752	T	C	5.26	2.99	1.00	-0.10	0.14	1.80	26.32	29.87	0.95	-0.27	0.20	0.84	68.42	67.15	1.00	-0.217	0.239	
rs9811423	C	G	36.84	37.30	1.00	-0.24	0.22	0.98	47.37	44.79	1.00	-0.23	0.28	1.11	15.79	17.90	1.00	-0.209	0.171	
rs1693703	T	C	73.68	51.03	0.10	-0.01	0.46	2.69	21.05	40.61	0.15	-0.42	0.03	0.39	5.26	7.17	1.00	-0.142	0.105	
rs7841649	T	C	0.00	0.00	1.00	0.00	0.00	0.00	15.79	2.39	0.01	-0.06	0.33	7.66	84.21	96.42	0.06	-0.316	0.071	
rs76592289	A	G	0.00	0.30	1.00	-0.02	0.01	0.00	0.00	8.65	0.37	-0.15	-0.02	0.00	100.00	91.05	0.35	0.027	0.154	
rs76557252	T	G	0.00	0.00	1.00	0.00	0.00	0.00	15.79	0.89	0.00	-0.04	0.34	20.77	84.21	99.11	0.00	-0.343	0.043	
rs506757	T	G	52.63	43.59	0.60	-0.17	0.35	1.44	42.11	47.75	0.81	-0.31	0.20	0.80	5.26	8.35	0.97	-0.164	0.104	
rs62166939	A	C	5.26	3.29	1.00	-0.10	0.14	1.63	26.32	34.93	0.61	-0.32	0.15	0.67	68.42	59.69	0.61	-0.160	0.332	

Table 6.7 SNPs associated with KCNJ11

101 unique SNPs associated with KCNJ11 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$.

			% AA						% AB						% BB					
dbSNP RS ID	Minor allele (A)	Major allele (B)	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio
rs78082945	T	C	94.74	93.43	1.00	-0.11	0.13	1.26	5.26	6.57	1.00	-0.13	0.11	0.79	0.00	0.00	1.00	0.000	0.000	0.000
rs62272114	A	G	0.00	0.00	1.00	0.00	0.00	0.00	0.00	5.08	0.66	-0.11	0.00	0.00	100.00	94.63	0.62	-0.001	0.111	0.000
rs112288451	T	C	100.00	94.92	0.66	0.00	0.11	0.00	0.00	2.99	0.97	-0.08	0.02	0.00	0.00	0.00	1.00	0.000	0.000	0.000
rs74806059	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	3.59	0.86	-0.09	0.02	0.00	100.00	96.41	0.86	-0.016	0.086	0.000
rs79876945	A	G	0.00	0.00	1.00	0.00	0.00	0.00	10.53	5.97	0.76	-0.12	0.22	1.85	89.47	94.03	0.76	-0.215	0.123	0.540
rs114687906	A	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	3.88	0.81	-0.09	0.01	0.00	94.74	95.22	1.00	-0.115	0.104	0.903
rs115477611	T	C	0.00	0.00	1.00	0.00	0.00	0.00	10.53	3.59	0.38	-0.10	0.24	3.16	89.47	96.41	0.98	-0.238	0.098	0.317
rs78771971	T	C	100.00	99.40	1.00	-0.01	0.03	0.00	0.00	0.60	1.00	-0.03	0.01	0.00	0.00	0.00	1.00	0.000	0.000	0.000
rs78854530	A	G	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.79	1.00	-0.06	0.02	0.00	100.00	98.21	1.00	-0.017	0.056	0.000
rs62503295	T	C	84.21	85.68	1.00	-0.20	0.17	0.89	10.53	14.03	0.93	-0.21	0.14	0.72	0.00	0.30	1.00	-0.016	0.008	0.000
rs114756881	T	C	94.74	85.66	0.44	-0.05	0.23	3.01	0.00	8.96	0.35	-0.15	-0.03	0.00	0.00	0.00	1.00	0.000	0.000	0.000
rs4498896	T	C	21.05	14.34	0.65	-0.15	0.29	1.59	36.84	45.36	0.63	-0.34	0.17	0.70	42.11	40.31	1.00	-0.230	0.269	1.077
rs79035182	T	G	0.00	0.00	1.00	0.00	0.00	0.00	0.00	3.88	0.86	-0.09	0.01	0.00	100.00	96.12	0.80	-0.013	0.092	0.000
rs74898314	A	G	0.00	0.30	1.00	-0.02	0.01	0.00	0.00	3.58	0.81	-0.09	0.02	0.00	100.00	96.12	0.80	-0.013	0.092	0.000
rs28445507	A	C	5.26	4.48	1.00	-0.10	0.12	1.18	47.37	36.71	0.50	-0.15	0.37	1.55	47.37	58.51	0.48	-0.374	0.148	0.638
rs55851518	A	G	0.00	0.30	1.00	-0.02	0.01	0.00	15.79	10.14	0.70	-0.14	0.25	1.66	84.21	89.56	0.73	-0.248	0.145	0.622
rs79263726	A	C	100.00	95.52	0.73	-0.01	0.10	0.00	0.00	4.48	0.73	-0.10	0.01	0.00	0.00	0.00	1.00	0.000	0.000	0.000
rs10898365	T	G	5.26	8.65	0.93	-0.17	0.10	0.59	31.58	36.13	0.88	-0.29	0.20	0.82	63.16	55.21	0.66	-0.173	0.334	1.391
rs66546253	A	G	21.05	12.86	0.51	-0.14	0.30	1.81	21.05	22.96	1.00	-0.23	0.19	0.89	57.89	63.58	0.80	-0.313	0.203	0.788
rs783307	T	C	84.21	71.33	0.35	-0.07	0.33	2.14	15.79	26.28	0.46	-0.31	0.10	0.53	0.00	2.39	1.00	-0.066	0.019	0.000
rs17734780	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	2.39	1.00	-0.07	0.02	0.00	100.00	97.61	1.00	-0.019	0.066	0.000
rs10432095	A	C	73.68	71.05	1.00	-0.21	0.26	1.14	26.32	27.46	1.00	-0.23	0.21	0.94	0.00	1.49	1.00	-0.047	0.015	0.000
rs75107770	A	G	0.00	0.00	1.00	0.00	0.00	0.00	5.26	4.48	1.00	-0.10	0.12	1.19	94.74	95.52	1.00	-0.122	0.103	0.844
rs13061963	T	G	5.26	0.00	0.09	-0.08	0.18	0.00	10.53	10.75	1.00	-0.15	0.14	0.98	84.21	89.25	0.77	-0.248	0.145	0.642
rs60400172	T	G	5.26	4.77	1.00	-0.10	0.11	1.11	0.00	3.88	0.81	-0.09	0.01	0.00	94.74	91.35	0.93	-0.100	0.168	1.705
rs76451747	A	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.20	1.00	-0.04	0.01	0.00	100.00	98.80	1.00	-0.013	0.037	0.000
rs12520495	A	G	47.37	45.96	1.00	-0.23	0.26	1.06	36.84	45.97	0.60	-0.35	0.16	0.69	15.79	8.07	0.46	-0.116	0.275	2.137
rs13183249	A	G	5.26	3.89	1.00	-0.10	0.13	1.37	36.84	39.70	1.00	-0.28	0.22	0.89	57.89	56.41	1.00	-0.230	0.262	1.062
rs72725509	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.19	1.00	-0.04	0.01	0.00	100.00	98.81	1.00	-0.013	0.037	0.000
rs80184435	A	G	0.00	0.00	1.00	0.00	0.00	0.00	5.26	5.07	1.00	-0.10	0.11	1.04	94.74	93.43	1.00	-0.105	0.134	1.265
rs62404592	A	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	2.39	1.00	-0.07	0.02	0.00	100.00	95.52	0.73	-0.010	0.097	0.000
rs117910348	A	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	2.68	1.00	-0.08	0.02	0.00	100.00	97.32	1.00	-0.020	0.075	0.000
rs79853069	A	G	63.16	82.39	0.08	-0.44	0.06	0.37	36.84	16.41	0.05	-0.05	0.45	2.97	0.00	0.30	1.00	-0.016	0.008	0.000

Table 6.7 SNPs associated with KCNJ11 (continued)

101 unique SNPs associated with KCNJ11 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

			% AA						% AB						% BB					
dbSNP RS ID	Minor allele (A)	Major allele (B)	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ra
rs71521249	A	G	100.00	98.51	1.00	-0.02	0.05	0.00	0.00	1.49	1.00	-0.05	0.02	0.00	0.00	0.00	1.00	0.000	0.000	0.000
rs114234140	A	G	0.00	0.00	1.00	0.00	0.00	0.00	5.26	3.59	1.00	-0.10	0.14	1.49	94.74	96.41	0.49	-0.137	0.103	0.670
rs114249268	A	G	0.00	4.49	1.00	-0.10	0.01	0.00	0.00	2.38	1.00	-0.07	0.02	0.00	100.00	85.95	0.16	0.071	0.213	0.000
rs112623189	A	G	0.00	0.00	1.00	0.00	0.00	0.00	21.05	7.76	0.12	-0.08	0.35	3.17	78.95	92.24	0.12	-0.346	0.083	0.316
rs115010323	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	2.09	1.00	-0.06	0.02	0.00	100.00	97.61	1.00	-0.019	0.066	0.000
rs116250326	T	C	0.00	0.00	1.00	0.00	0.00	0.00	5.26	0.90	0.55	-0.08	0.17	6.13	94.74	98.80	0.67	-0.170	0.089	0.218
rs77111308	T	C	0.00	0.00	1.00	0.00	0.00	0.00	5.26	11.64	0.64	-0.20	0.07	0.42	94.74	88.36	0.64	-0.071	0.202	2.372
rs77823977	T	C	100.00	96.42	0.86	-0.02	0.09	0.00	0.00	3.58	0.86	-0.09	0.02	0.00	0.00	0.00	1.00	0.000	0.000	0.000
rs78374474	T	C	0.00	0.30	1.00	-0.02	0.01	0.00	15.79	2.99	0.03	-0.07	0.32	6.08	84.21	96.71	0.04	-0.320	0.067	0.181
rs192657690	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.49	1.00	-0.05	0.02	0.00	100.00	98.51	1.00	-0.015	0.047	0.000
rs116983235	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.60	1.00	-0.03	0.01	0.00	100.00	99.40	1.00	-0.011	0.027	0.000
rs10846754	A	G	0.00	3.58	0.86	-0.09	0.02	0.00	26.32	31.95	0.80	-0.29	0.18	0.76	73.68	64.47	0.57	-0.144	0.326	1.543
rs576184	A	C	5.26	15.22	0.39	-0.24	0.04	0.31	63.16	46.56	0.25	-0.09	0.42	1.97	31.58	38.21	0.74	-0.312	0.180	0.746
rs16951460	A	G	100.00	92.23	0.42	0.02	0.14	0.00	0.00	3.88	0.80	-0.09	0.01	0.00	0.00	1.20	1.00	-0.037	0.013	0.000
rs3748592	A	G	0.00	0.00	1.00	0.00	0.00	0.00	10.53	13.72	0.96	-0.21	0.14	0.74	89.47	86.28	0.96	-0.140	0.205	1.352
rs2096376	T	C	63.16	52.53	0.51	-0.15	0.36	1.55	36.84	37.33	1.00	-0.24	0.22	0.98	0.00	10.14	0.29	-0.168	-0.037	0.000
rs32555	A	G	68.42	68.36	1.00	-0.22	0.22	1.00	31.58	26.87	0.86	-0.20	0.29	1.26	0.00	4.17	0.77	-0.097	0.010	0.000
rs9261089	T	C	89.47	92.53	0.97	-0.20	0.14	0.69	10.53	7.17	0.93	-0.14	0.20	1.52	0.00	0.00	1.00	0.000	0.000	0.000
rs28400886	A	G	0.00	0.30	1.00	-0.02	0.01	0.00	5.26	9.26	0.86	-0.18	0.09	0.54	94.74	90.44	0.83	-0.093	0.177	1.902
rs4959042	T	C	100.00	98.81	1.00	-0.01	0.04	0.00	0.00	1.19	1.00	-0.04	0.01	0.00	0.00	0.00	1.00	0.000	0.000	0.000
rs34704616	A	G	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.60	1.00	-0.03	0.01	0.00	100.00	99.40	1.00	-0.011	0.027	0.000

Table 6.7 SNPs associated with KCNJ11 (continued)

101 unique SNPs associated with KCNJ11 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

			% AA						% AB						% BB					
dbSNP RS ID	Minor allele (A)	Major allele (B)	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Rat
rs1573298	C	G	5.26	6.87	1.00	-0.13	0.11	0.75	47.37	37.33	0.53	-0.16	0.36	1.51	47.37	55.21	0.69	-0.338	0.183	0.730
rs397596	T	C	100.00	100.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.000	0.000	0.000
rs139080520	A	G	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	100.00	100.00	1.00	0.000	0.000	0.000
rs13268757	A	G	5.26	2.39	0.99	-0.10	0.16	2.27	15.79	26.57	0.44	-0.31	0.10	0.52	78.95	71.04	0.64	-0.139	0.301	1.528
rs114814001	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	5.08	0.66	-0.11	0.00	0.00	100.00	94.92	0.66	-0.004	0.107	0.000
rs79765628	T	C	0.00	0.00	1.00	0.00	0.00	0.00	5.26	5.08	1.00	-0.10	0.11	1.04	94.74	94.92	1.00	-0.107	0.104	0.963
rs117389196	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	3.87	0.81	-0.09	0.01	0.00	100.00	95.83	0.77	-0.010	0.097	0.000
rs141834826	A	G	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	100.00	99.70	1.00	-0.008	0.016	0.000
rs201447432	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	2.69	1.00	-0.08	0.02	0.00	100.00	96.41	0.86	-0.016	0.086	0.000
rs146548891	T	G	100.00	99.11	1.00	-0.01	0.03	0.00	0.00	0.60	1.00	-0.03	0.01	0.00	0.00	0.00	1.00	0.000	0.000	0.000
rs142720326	A	G	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.60	1.00	-0.03	0.01	0.00	100.00	99.40	1.00	-0.011	0.027	0.000
rs201960928	A	G	0.00	0.00	1.00	0.00	0.00	0.00	0.00	2.10	1.00	-0.06	0.02	0.00	100.00	97.60	1.00	-0.019	0.066	0.000
rs139889337	A	G	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.79	1.00	-0.06	0.02	0.00	100.00	97.91	1.00	-0.017	0.056	0.000
rs113993403	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.79	1.00	-0.06	0.02	0.00	100.00	98.21	1.00	-0.017	0.056	0.000
rs138962514	T	C	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	100.00	100.00	1.00	0.000	0.000	0.000

Table 6.7 SNPs associated with KCNJ11 (final)

101 unique SNPs associated with KCNJ11 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at *P*<0.05

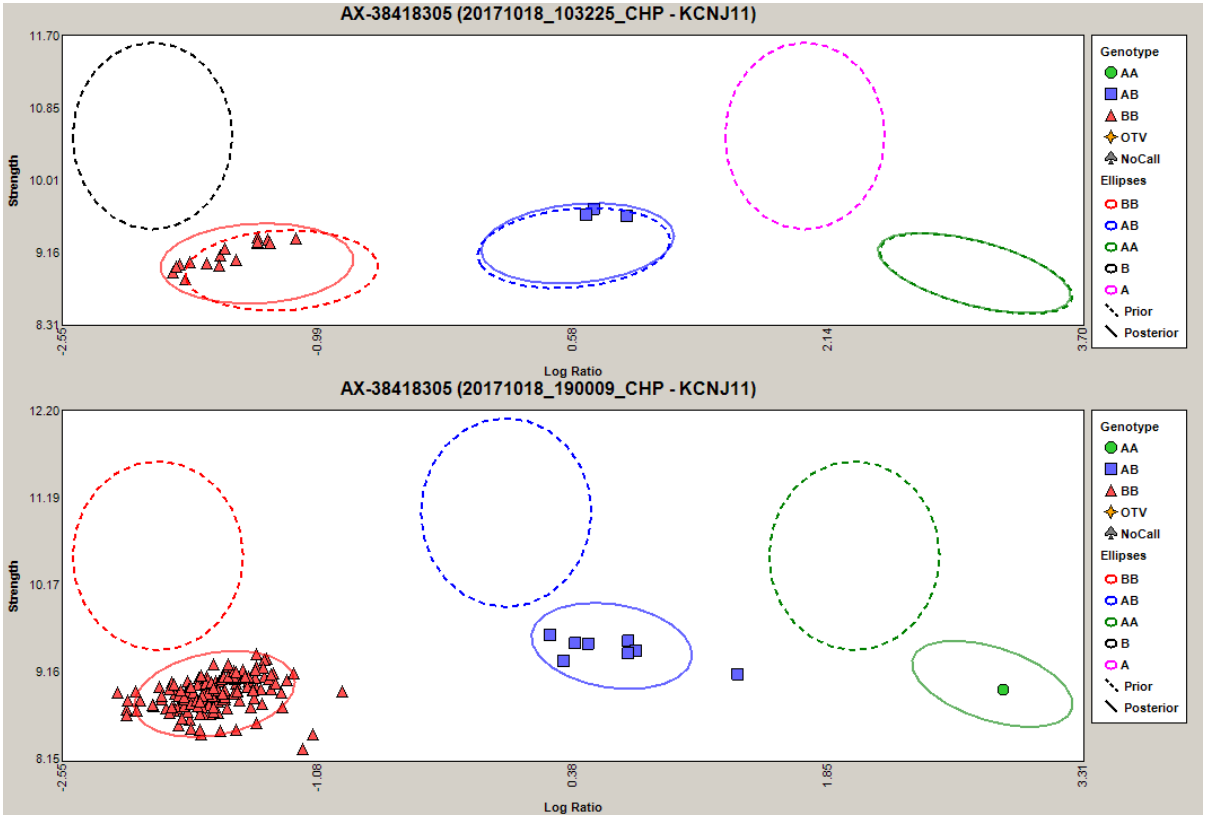


Figure 6.1 Clustering analysis for KCNJ11- rs78374474

Clustering of genotypes between the control (top panel) and DIASTRAT (bottom panel) cohorts shown graphically for rs78374474

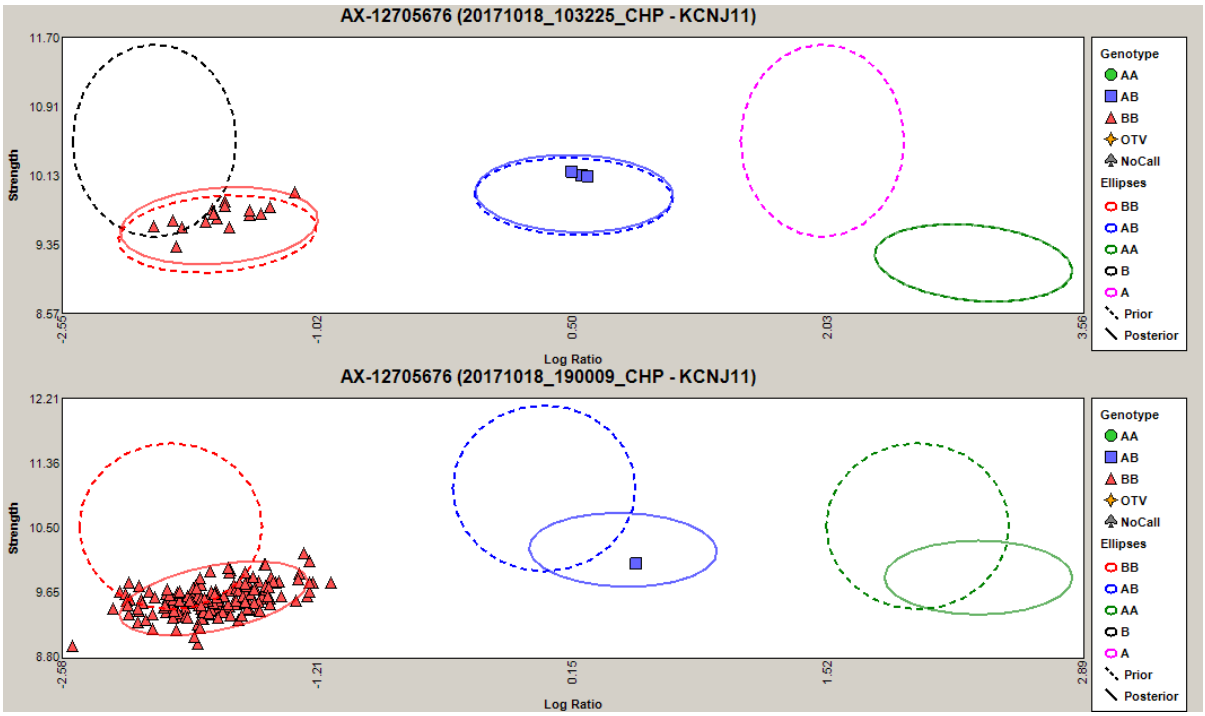


Figure 6.2 Clustering analysis for KCNJ11- rs76557252

Clustering of genotypes between the control (top panel) and DIASTRAT (bottom panel) cohorts shown graphically for rs76557252

			% AA						% AB						% BB					
dbSNP RS ID	Minor allele (A)	Major allele (B)	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio
rs16841998	T	C	63.16	73.44	0.514	-0.356	0.147	0.620	21.05	15.83	0.804	-0.164	0.270	1.418	0.00	0.00	1.000	0.000	0.000	0.000
rs17461843	T	C	100.00	88.36	0.233	0.050	0.186	0.000	0.00	8.95	0.352	-0.154	-0.027	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs17571372	T	C	0.00	0.60	1.000	-0.027	0.011	0.000	5.26	9.56	0.787	-0.177	0.093	0.525	94.74	89.84	0.704	-0.086	0.185	2.035
	T	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	99.70	1.000	-0.008	0.016	0.000
rs3006968	A	G	26.32	23.29	0.985	-0.202	0.264	1.176	47.37	47.75	1.000	-0.238	0.233	0.985	26.32	28.66	1.000	-0.254	0.206	0.889
rs6039591	A	G	100.00	95.22	0.145	-0.007	0.102	0.000	0.00	4.78	0.145	-0.102	0.007	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs6883010	A	G	5.26	18.81	0.059	-0.276	0.003	0.240	52.63	45.66	0.727	-0.191	0.331	1.322	42.11	35.53	0.749	-0.191	0.325	1.320
rs6133707	A	G	52.63	60.90	0.646	-0.345	0.177	0.713	47.37	34.02	0.374	-0.125	0.395	1.745	0.00	4.78	0.145	-0.102	0.007	0.000
rs77707280	A	G	0.00	0.60	1.000	-0.027	0.011	0.000	10.53	10.14	1.000	-0.143	0.149	1.042	89.47	88.96	1.000	-0.143	0.153	1.055
rs807936	T	C	42.11	37.92	0.908	-0.215	0.301	1.191	52.63	44.47	0.670	-0.180	0.342	1.387	5.26	17.61	0.092	-0.264	0.015	0.260
rs114348392	T	G	0.00	0.00	1.000	0.000	0.000	0.000	5.26	2.98	1.000	-0.103	0.145	1.807	89.47	96.72	0.535	-0.242	0.094	0.289
rs9356928	A	G	15.79	22.68	0.643	-0.271	0.130	0.639	52.63	47.17	0.825	-0.207	0.315	1.244	31.58	29.56	1.000	-0.216	0.257	1.100
rs9460973	A	T	0.00	0.00	1.000	0.000	0.000	0.000	0.00	8.95	0.352	-0.154	-0.027	0.000	100.00	90.75	0.336	0.030	0.159	0.000
rs58554303	T	C	94.74	94.33	1.000	-0.104	0.109	1.082	5.26	5.37	1.000	-0.109	0.104	0.979	0.00	0.00	1.000	0.000	0.000	0.000
rs920628	A	G	94.74	92.84	1.000	-0.105	0.142	1.389	5.26	6.57	1.000	-0.134	0.105	0.790	0.00	0.60	1.000	-0.027	0.011	0.000
rs16873732	T	C	94.74	92.84	1.000	-0.105	0.142	1.389	5.26	6.57	1.000	-0.134	0.105	0.790	0.00	0.60	1.000	-0.027	0.011	0.000
rs1041791	T	C	57.89	65.08	0.710	-0.329	0.187	0.738	42.11	31.93	0.530	-0.155	0.360	1.550	0.00	2.68	1.000	-0.075	0.020	0.000
rs61741363	A	G	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs113319303	A	G	0.00	2.69	1.000	-0.075	0.020	0.000	0.00	3.28	0.686	-0.081	0.018	0.000	100.00	93.73	0.537	0.005	0.121	0.000
rs116581086	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.89	1.000	-0.027	0.011	0.000	100.00	99.11	1.000	-0.011	0.027	0.000
rs74745471	A	G	0.00	0.00	1.000	0.000	0.000	0.000	5.26	11.34	0.554	-0.198	0.074	0.434	94.74	88.66	0.554	-0.074	0.198	2.303
rs61936141	T	C	100.00	96.11	0.145	-0.013	0.092	0.000	0.00	3.59	0.686	-0.086	0.016	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs79090092	A	G	0.00	0.30	1.000	-0.016	0.008	0.000	5.26	7.17	1.000	-0.142	0.105	0.719	94.74	92.53	1.000	-0.105	0.150	1.453
rs10905750	A	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	4.78	0.145	-0.102	0.007	0.000	100.00	95.22	0.145	-0.007	0.102	0.000
rs231847	A	G	5.26	12.54	0.421	-0.210	0.063	0.387	52.63	56.42	0.935	-0.298	0.224	0.858	42.11	31.03	0.480	-0.147	0.367	1.616
rs129072	T	C	0.00	3.58	0.519	-0.086	0.016	0.000	15.79	27.46	0.318	-0.319	0.084	0.495	84.21	68.66	0.151	-0.045	0.359	2.435
rs75898263	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000

Table 6.8 SNPs associated with KCNQ1

68 unique SNPs associated with KCNQ1 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

			% AA						% AB						% BB					
dbSNP RS ID	Minor allele (A)	Major allele (B)	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Rat
rs111871569	A	G	0.00	0.30	1.000	-0.016	0.008	0.000	10.53	6.86	0.907	-0.131	0.208	1.597	89.47	92.24	1.000	-0.195	0.142	0.715
rs75875515	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	4.18	0.145	-0.097	0.010	0.000	100.00	95.22	0.145	-0.007	0.102	0.000
rs7156987	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	3.58	0.686	-0.086	0.016	0.000	100.00	94.64	0.145	-0.001	0.111	0.000
rs55767542	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	4.18	0.145	-0.097	0.010	0.000	100.00	95.22	0.145	-0.007	0.102	0.000
rs35162844	A	G	0.00	0.60	1.000	-0.027	0.011	0.000	15.79	6.57	0.453	-0.104	0.286	2.667	84.21	92.54	0.519	-0.278	0.112	0.430
rs73063633	T	G	94.74	94.92	1.000	-0.107	0.104	0.962	5.26	4.78	1.000	-0.104	0.115	1.108	0.00	0.00	1.000	0.000	0.000	0.000
rs116045044	T	G	94.74	91.05	0.874	-0.097	0.173	1.769	5.26	8.06	1.000	-0.158	0.106	0.634	0.00	0.00	1.000	0.000	0.000	0.000
rs77692939	A	G	100.00	96.41	0.145	-0.016	0.086	0.000	0.00	1.49	1.000	-0.047	0.015	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs72811968	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	3.28	0.145	-0.081	0.018	0.000	100.00	96.72	0.686	-0.018	0.081	0.000
rs76238538	T	C	100.00	94.63	0.145	-0.001	0.111	0.000	0.00	5.37	0.072	-0.111	0.001	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs79931282	A	G	89.47	92.84	0.940	-0.204	0.135	0.656	10.53	6.57	0.875	-0.131	0.208	1.674	0.00	0.00	1.000	0.000	0.000	0.000
rs9467059	A	G	100.00	88.95	0.255	0.043	0.177	0.000	0.00	10.15	0.292	-0.168	-0.037	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs188539852	T	C	0.00	0.00	1.000	0.000	0.000	0.000	5.26	2.38	0.992	-0.101	0.159	2.274	94.74	97.62	0.992	-0.159	0.101	0.440
rs115704940	T	C	0.00	0.30	1.000	-0.016	0.008	0.000	5.26	5.37	1.000	-0.109	0.104	0.978	94.74	94.33	1.000	-0.104	0.109	1.083
rs116858972	T	C	0.00	0.00	1.000	0.000	0.000	0.000	5.26	3.88	1.000	-0.103	0.130	1.376	94.74	96.12	1.000	-0.130	0.103	0.727
rs117103115	A	G	100.00	90.14	0.306	0.034	0.163	0.000	0.00	5.97	0.072	-0.116	-0.002	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs77936291	A	G	94.74	94.32	1.000	-0.104	0.109	1.084	5.26	5.68	1.000	-0.109	0.104	0.922	0.00	0.00	1.000	0.000	0.000	0.000
rs79947987	T	C	100.00	99.11	1.000	-0.011	0.027	0.000	0.00	0.60	1.000	-0.027	0.011	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs78468689	A	G	0.00	0.00	1.000	0.000	0.000	0.000	5.26	7.46	1.000	-0.150	0.105	0.689	94.74	92.54	1.000	-0.105	0.150	1.452
rs80140016	T	G	0.00	0.00	1.000	0.000	0.000	0.000	10.53	7.76	0.875	-0.142	0.195	1.398	89.47	91.94	1.000	-0.195	0.142	0.745
rs75894327	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	7.16	0.465	-0.131	-0.011	0.000	100.00	92.84	0.465	0.011	0.131	0.000

Table 6.8 SNPs associated with KCNQ1 (continued)

68 unique SNPs associated with KCNQ1 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

			% AA					% AB					% BB				
dbSNP RS ID	Minor allele (A)	Major allele (B)	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio
rs114351051	T	C	100.00	84.22	0.124	0.084	0.231	0.000	0.00	9.53	0.321	-0.159	-0.030	0.000	0.00	0.89	1.000	-0.027	0.011	0.000
rs115005266	T	C	0.00	0.00	1.000	0.000	0.000	0.000	5.26	4.48	1.000	-0.103	0.122	1.185	94.74	94.93	1.000	-0.107	0.104	0.962
rs112214748	A	G	0.00	0.00	1.000	0.000	0.000	0.000	26.32	5.38	0.076	-0.020	0.436	6.283	73.68	94.62	0.076	-0.436	0.020	0.159
rs7119884	A	G	94.74	93.14	1.000	-0.105	0.134	1.325	5.26	6.86	1.000	-0.134	0.105	0.755	0.00	0.00	1.000	0.000	0.000	0.000
rs6057010	A	G	89.47	87.15	1.000	-0.144	0.193	1.253	10.53	12.85	1.000	-0.193	0.144	0.798	0.00	0.00	1.000	0.000	0.000	0.000
rs4690087	A	G	0.00	5.39	0.071	-0.111	0.001	0.000	26.32	3.87	0.054	-0.004	0.452	8.869	73.68	90.74	0.166	-0.398	0.061	0.286
rs11754464	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	99.70	1.000	-0.008	0.016	0.000
rs707938	A	G	42.11	41.78	1.000	-0.230	0.238	1.014	47.37	46.29	1.000	-0.233	0.251	1.044	10.53	11.33	1.000	-0.161	0.143	0.920
rs35185125	A	C	0.00	0.00	1.000	0.000	0.000	0.000	5.26	1.19	0.810	-0.089	0.170	4.597	94.74	98.81	0.810	-0.170	0.089	0.218
rs28381355	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	1.20	1.000	-0.037	0.013	0.000	100.00	98.80	1.000	-0.013	0.037	0.000
rs183314661	A	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	4.78	0.145	-0.102	0.007	0.000	100.00	95.22	0.145	-0.007	0.102	0.000
rs77320475	A	G	0.00	0.00	1.000	0.000	0.000	0.000	15.79	7.16	0.496	-0.108	0.282	2.430	84.21	92.54	0.519	-0.278	0.112	0.430
rs17812699	A	G	0.00	0.30	1.000	-0.016	0.008	0.000	0.00	15.23	0.135	-0.226	-0.081	0.000	100.00	84.47	0.129	0.081	0.226	0.000
rs199853687	A	C	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs115491500	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs11553182	T	C	0.00	0.00	1.000	0.000	0.000	0.000	15.79	17.90	1.000	-0.209	0.171	0.860	84.21	82.10	1.000	-0.171	0.209	1.163
	C	G	100.00	99.70	1.000	-0.008	0.016	0.000	0.00	0.30	1.000	-0.016	0.008	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs341047	A	G	0.00	0.60	1.000	-0.027	0.011	0.000	31.58	20.31	0.441	-0.132	0.354	1.811	68.42	78.50	0.509	-0.343	0.144	0.594
rs150934987	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs139577182	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.89	1.000	-0.027	0.011	0.000	100.00	99.11	1.000	-0.011	0.027	0.000

Table 6.8 SNPs associated with KCNQ1 (final)

68 unique SNPs associated with KCNQ1 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

			% AA					% AB					% BB				
dbSNP RS ID	Minor allele (A)	Major allele (B)	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI	Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI	Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI	Odds Rati
rs11755881	A	G	0.00	1.49	1.000	-0.047 0.015	0.000	5.26	17.02	0.113	-0.255 0.022	0.271	94.74	81.49	0.066	-0.007 0.272	4.089
rs1861	A	C	0.00	0.30	1.000	-0.016 0.008	0.000	5.26	4.47	1.000	-0.103 0.122	1.186	94.74	95.23	1.000	-0.115 0.104	0.902
rs28568667	T	C	63.16	73.11	0.533	-0.353 0.151	0.630	26.32	23.90	1.000	-0.205 0.251	1.137	0.00	0.00	1.000	0.000 0.000	0.000
rs4770748	A	G	0.00	0.30	1.000	-0.016 0.008	0.000	0.00	13.44	0.177	-0.204 -0.064	0.000	100.00	86.26	0.169	0.067 0.208	0.000
rs663824	A	G	36.84	42.39	0.813	-0.310 0.197	0.793	42.11	43.88	1.000	-0.262 0.230	0.930	21.05	13.73	0.640	-0.144 0.289	1.675
rs6905159	A	G	15.79	25.66	0.424	-0.299 0.103	0.543	57.89	49.55	0.639	-0.176 0.342	1.400	26.32	24.19	1.000	-0.205 0.251	1.119
rs888576	T	C	0.00	0.60	1.000	-0.027 0.011	0.000	31.58	16.40	0.258	-0.092 0.393	2.353	68.42	83.00	0.282	-0.389 0.096	0.444
rs13325508	T	C	94.74	95.23	1.000	-0.115 0.104	0.902	5.26	4.77	1.000	-0.104 0.115	1.108	0.00	0.00	1.000	0.000 0.000	0.000
rs55723035	T	C	0.00	0.00	1.000	0.000 0.000	0.000	0.00	2.99	0.887	-0.081 0.018	0.000	100.00	97.01	0.880	-0.018 0.081	0.000
rs77356515	T	G	0.00	0.00	1.000	0.000 0.000	0.000	10.53	1.79	0.404	-0.082 0.253	6.444	89.47	98.21	0.404	-0.253 0.082	0.155
rs75363174	A	G	0.00	0.30	1.000	-0.016 0.008	0.000	10.53	11.04	1.000	-0.153 0.143	0.948	89.47	88.66	1.000	-0.143 0.161	1.087
rs4751674	T	C	21.05	11.64	0.491	-0.123 0.308	2.023	36.84	37.31	1.000	-0.236 0.225	0.980	42.11	50.75	0.621	-0.345 0.172	0.706
rs61433965	A	G	100.00	96.72	0.081	-0.018 0.081	0.000	0.00	2.98	0.887	-0.081 0.018	0.000	0.00	0.00	1.000	0.000 0.000	0.000
rs58838391	A	G	0.00	0.00	1.000	0.000 0.000	0.000	0.00	2.99	0.887	-0.081 0.018	0.000	100.00	97.01	0.880	-0.018 0.081	0.000
rs76280424	A	G	0.00	0.00	1.000	0.000 0.000	0.000	0.00	0.00	1.000	0.000 0.000	0.000	100.00	100.00	1.000	0.000 0.000	0.000
rs77794667	T	C	0.00	0.00	1.000	0.000 0.000	0.000	5.26	3.28	1.000	-0.103 0.145	1.637	94.74	96.72	1.000	-0.145 0.103	0.611
rs59559871	T	C	0.00	0.00	1.000	0.000 0.000	0.000	0.00	0.00	1.000	0.000 0.000	0.000	100.00	99.70	1.000	-0.008 0.016	0.000
rs72826927	A	G	0.00	0.30	1.000	-0.016 0.008	0.000	10.53	13.13	1.000	-0.193 0.144	0.778	89.47	86.57	1.000	-0.144 0.201	1.319
rs945094	A	T	0.00	0.00	1.000	0.000 0.000	0.000	5.26	5.08	1.000	-0.104 0.107	1.037	94.74	94.92	1.000	-0.107 0.104	0.964
rs72751116	A	G	100.00	99.40	1.000	-0.011 0.027	0.000	0.00	0.60	1.000	-0.027 0.011	0.000	0.00	0.00	1.000	0.000 0.000	0.000
rs59706474	T	C	0.00	0.00	1.000	0.000 0.000	0.000	5.26	3.28	1.000	-0.103 0.145	1.639	94.74	96.72	1.000	-0.145 0.103	0.610
rs73251982	A	G	0.00	1.80	1.000	-0.056 0.017	0.000	15.79	2.09	0.196	-0.055 0.331	8.805	84.21	94.32	0.391	-0.297 0.092	0.321

Table 6.9 SNPs associated with HNF1α

38 unique SNPs associated with HNF1α were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

dbSNP RS ID	Minor allele (A)	Major allele (B)	% AA						% AB						% BB					
			Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio
rs72489176	A	G	57.89	62.39	0.887	-0.301	0.215	0.829	36.84	32.55	0.898	-0.211	0.294	1.209	5.26	5.07	1.000	-0.104	0.107	1.041
rs77367389	A	C	94.74	98.51	0.856	-0.167	0.093	0.272	5.26	1.49	0.856	-0.093	0.167	3.673	0.00	0.00	1.000	0.000	0.000	0.000
rs73668591	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	1.79	1.000	-0.056	0.017	0.000	100.00	97.91	1.000	-0.017	0.056	0.000
rs73669423	T	C	84.21	84.78	1.000	-0.179	0.170	0.957	15.79	15.22	1.000	-0.170	0.179	1.045	0.00	0.00	1.000	0.000	0.000	0.000
rs62493228	A	C	0.00	0.00	1.000	0.000	0.000	0.000	15.79	10.45	0.770	-0.145	0.248	1.607	84.21	89.55	0.770	-0.248	0.145	0.622
rs76569571	T	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	4.47	0.205	-0.097	0.010	0.000	100.00	93.74	0.538	0.005	0.121	0.000
rs80312260	A	G	0.00	0.00	1.000	0.000	0.000	0.000	10.53	3.58	0.564	-0.098	0.238	3.167	89.47	96.42	0.564	-0.238	0.098	0.316
rs2244608	A	G	36.84	52.25	0.274	-0.409	0.099	0.533	63.16	39.39	0.068	-0.015	0.491	2.638	0.00	8.36	0.385	-0.145	-0.021	0.000
rs11616995	A	G	100.00	96.72	0.686	-0.018	0.081	0.000	0.00	3.28	0.686	-0.081	0.018	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs11647932	T	C	0.00	0.89	1.000	-0.027	0.011	0.000	42.11	19.71	0.092	-0.031	0.480	2.962	57.89	79.09	0.114	-0.468	0.043	0.363
rs8102561	T	C	63.16	70.16	0.715	-0.322	0.183	0.729	31.58	27.16	0.885	-0.200	0.288	1.238	5.26	2.69	1.000	-0.102	0.153	2.012
rs340141	T	C	10.53	10.74	1.000	-0.145	0.143	0.977	42.11	44.46	1.000	-0.278	0.230	0.908	47.37	44.49	0.997	-0.232	0.290	1.123
rs150333766	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.89	1.000	-0.027	0.011	0.000	100.00	99.11	1.000	-0.011	0.027	0.000
rs189023122	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs150428096	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	1.20	1.000	-0.037	0.013	0.000	100.00	97.90	1.000	-0.017	0.056	0.000
rs147799118	T	C	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000

Table 6.9 SNPs associated with HNF1α (final)

38 unique SNPs associated with HNF1α were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

			% AA						% AB						% BB					
dbSNP RS ID	Minor allele (A)	Major allele (B)	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio
rs12894204	A	G	0.00	3.57	0.524	-0.086	0.016	0.000	36.84	33.15	0.940	-0.215	0.290	1.176	63.16	63.27	1.000	-0.229	0.225	0.995
rs17605420	A	G	94.74	96.11	1.000	-0.130	0.103	0.729	5.26	3.89	1.000	-0.103	0.130	1.374	0.00	0.00	1.000	0.000	0.000	0.000
rs17763253	T	C	0.00	1.80	1.000	-0.056	0.017	0.000	10.53	6.25	0.840	-0.127	0.212	1.764	89.47	91.95	1.000	-0.195	0.142	0.744
rs6728493	A	C	47.37	51.94	0.883	-0.307	0.215	0.833	42.11	40.90	1.000	-0.230	0.253	1.051	10.53	7.16	0.940	-0.135	0.204	1.526
rs9906935	A	G	31.58	50.75	0.142	-0.438	0.054	0.448	57.89	39.10	0.174	-0.069	0.447	2.141	10.53	9.85	1.000	-0.143	0.156	1.077
rs7089262	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	7.76	0.423	-0.140	-0.017	0.000	100.00	91.94	0.404	0.017	0.140	0.000
rs76677854	T	C	84.21	91.04	0.640	-0.263	0.129	0.525	15.79	8.66	0.615	-0.125	0.267	1.978	0.00	0.00	1.000	0.000	0.000	0.000
rs145079521	A	C	89.47	93.13	0.908	-0.208	0.131	0.627	10.53	6.57	0.875	-0.131	0.208	1.673	0.00	0.00	1.000	0.000	0.000	0.000
rs78082945	T	C	94.74	93.43	1.000	-0.105	0.134	1.267	5.26	6.57	1.000	-0.134	0.105	0.791	0.00	0.00	1.000	0.000	0.000	0.000
rs62272114	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.08	0.102	-0.107	0.004	0.000	100.00	94.63	0.072	-0.001	0.111	0.000
rs77385406	A	G	0.00	0.00	1.000	0.000	0.000	0.000	21.05	5.97	0.192	-0.062	0.365	4.203	78.95	94.03	0.174	-0.365	0.062	0.238
rs61732118	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs61875109	A	C	21.05	5.37	0.174	-0.058	0.369	4.698	42.11	33.14	0.600	-0.167	0.348	1.467	36.84	61.19	0.061	-0.495	0.012	0.370
rs116369954	T	C	100.00	90.45	0.321	0.030	0.159	0.000	0.00	8.65	0.368	-0.149	-0.024	0.000	0.00	0.30	1.000	-0.016	0.008	0.000
rs79805154	A	G	89.47	85.96	0.926	-0.136	0.209	1.388	10.53	13.44	0.991	-0.201	0.144	0.758	0.00	0.30	1.000	-0.016	0.008	0.000
rs77064952	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	1.49	1.000	-0.047	0.015	0.000	100.00	97.91	1.000	-0.017	0.056	0.000
rs10059523	A	G	0.00	0.00	1.000	0.000	0.000	0.000	5.26	5.96	1.000	-0.117	0.105	0.876	94.74	94.04	1.000	-0.105	0.117	1.141
rs78183526	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.30	1.000	-0.016	0.008	0.000	100.00	99.70	1.000	-0.008	0.016	0.000
rs587667473	A	C	47.37	63.29	0.269	-0.421	0.100	0.522	52.63	36.41	0.258	-0.096	0.425	1.941	0.00	0.00	1.000	0.000	0.000	0.000
rs73179006	T	G	0.00	0.00	1.000	0.000	0.000	0.000	10.53	7.76	1.000	-0.142	0.195	1.399	89.47	92.24	1.000	-0.195	0.142	0.715
rs113262704	T	G	0.00	0.00	1.000	0.000	0.000	0.000	10.53	5.95	0.808	-0.123	0.215	1.859	89.47	93.75	0.840	-0.212	0.127	0.567
rs61989114	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	1.49	1.000	-0.047	0.015	0.000	100.00	98.51	1.000	-0.015	0.047	0.000

Table 6.10 SNPs associated with TCF7L2

44 unique SNPs associated with TCF7L2 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

			% AA						% AB						% BB					
dbSNP RS ID	Minor allele (A)	Major allele (B)	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio	Control (n=19)	DIASTRAT (n=254)	*p*-value	95% CI		Odds Ratio
rs55656505	T	C	100.00	91.64	0.385	0.021	0.145	0.000	0.00	8.06	0.404	-0.140	-0.017	0.000	0.00	0.30	1.000	-0.016	0.008	0.000
rs4783943	A	G	0.00	6.56	0.512	-0.126	-0.008	0.000	47.37	36.71	0.509	-0.153	0.368	1.551	52.63	56.73	0.915	-0.302	0.220	0.848
rs3026084	A	G	100.00	94.04	0.565	0.002	0.116	0.000	0.00	4.77	0.147	-0.102	0.007	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs189314829	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.37	0.072	-0.111	0.001	0.000	100.00	94.03	0.072	0.002	0.116	0.000
rs75107770	A	G	0.00	0.00	1.000	0.000	0.000	0.000	5.26	4.48	1.000	-0.103	0.122	1.185	94.74	95.52	1.000	-0.122	0.103	0.844
rs73223028	T	C	0.00	0.00	1.000	0.000	0.000	0.000	10.53	2.39	0.455	-0.086	0.249	4.809	89.47	97.61	0.455	-0.249	0.086	0.208
rs116929578	A	G	94.74	90.14	0.745	-0.089	0.181	1.970	5.26	8.96	0.872	-0.173	0.097	0.564	0.00	0.30	1.000	-0.016	0.008	0.000
rs117041486	A	G	0.00	0.00	1.000	0.000	0.000	0.000	10.53	2.09	0.455	-0.082	0.253	5.515	89.47	97.91	0.455	-0.253	0.082	0.181
rs79565172	A	G	94.74	97.62	0.992	-0.159	0.101	0.439	5.26	1.79	0.902	-0.097	0.163	3.049	0.00	0.00	1.000	0.000	0.000	0.000
rs117582734	T	C	0.00	0.00	1.000	0.000	0.000	0.000	15.79	11.05	0.824	-0.149	0.244	1.509	84.21	88.95	0.824	-0.244	0.149	0.662
rs12243326	T	C	47.37	39.71	0.684	-0.185	0.337	1.367	42.11	48.94	0.734	-0.326	0.192	0.759	10.53	11.35	1.000	-0.161	0.143	0.919
rs4889830	A	G	31.58	25.66	0.779	-0.184	0.304	1.337	42.11	51.65	0.568	-0.353	0.164	0.681	26.32	22.69	0.939	-0.198	0.268	1.217
rs3787493	A	G	21.05	24.17	0.976	-0.249	0.189	0.836	47.37	45.07	1.000	-0.233	0.282	1.097	31.58	30.45	1.000	-0.217	0.242	1.054
rs4713505	T	G	0.00	4.77	0.524	-0.102	0.007	0.000	73.68	30.75	0.000	0.196	0.664	6.306	26.32	64.48	0.002	-0.617	-0.148	0.197
rs2070600	T	C	0.00	0.00	1.000	0.000	0.000	0.000	36.84	13.73	0.072	-0.019	0.480	3.664	63.16	85.97	0.076	-0.476	0.023	0.280
rs3131300	A	G	52.63	56.72	0.524	-0.302	0.220	0.848	42.11	34.94	0.711	-0.187	0.329	1.354	5.26	8.05	1.000	-0.158	0.106	0.635
rs73471190	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.60	1.000	-0.027	0.011	0.000	100.00	98.81	1.000	-0.013	0.037	0.000
rs148924158	A	G	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs143828311	T	C	0.00	0.00	1.000	0.000	0.000	0.000	5.26	2.09	0.948	-0.097	0.163	2.604	94.74	97.91	0.948	-0.163	0.097	0.384
rs201967398	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs143652701	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs142236510	T	C	100.00	95.23	0.524	-0.007	0.102	0.000	0.00	4.77	0.147	-0.102	0.007	0.000	0.00	0.00	1.000	0.000	0.000	0.000

Table 6.10 SNPs associated with TCF7L2 (final)

44 unique SNPs associated with TCF7L2 were identified, and genotyped. Following a two portions t-test significantly different prevalence's are highlighted.

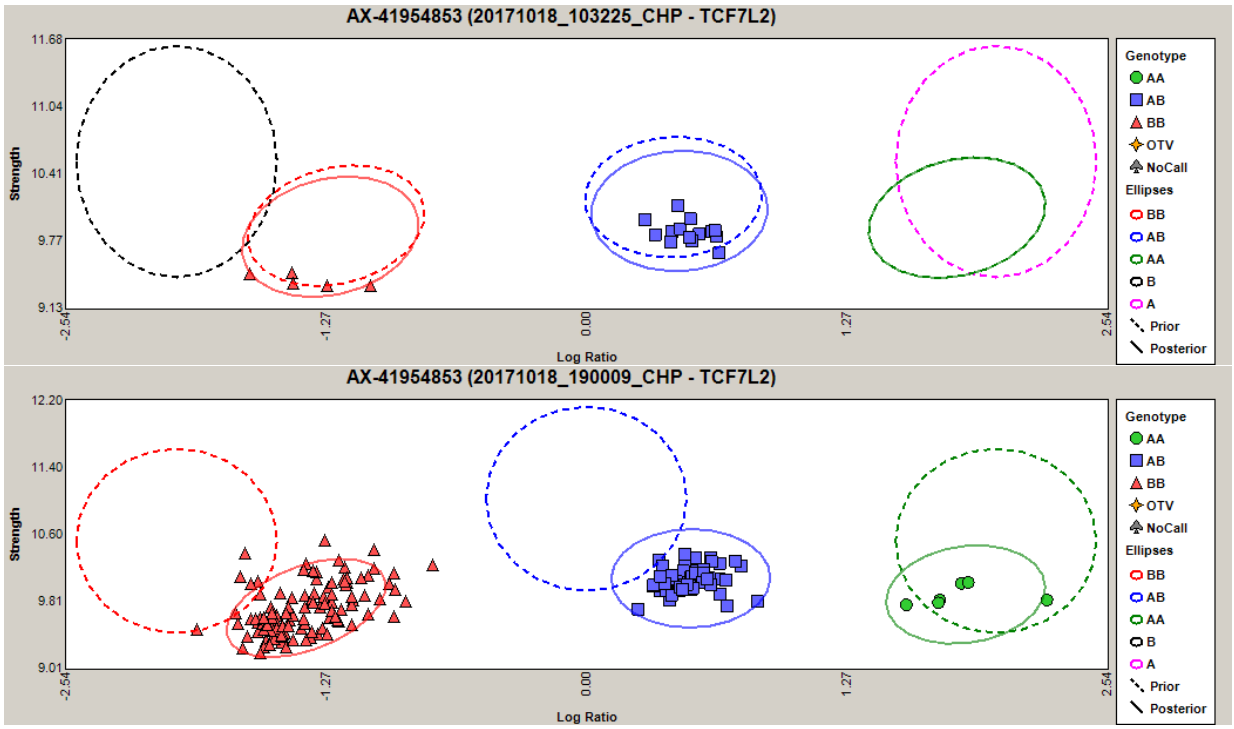


Figure 6.3 Clustering analysis for TCF7L2- rs4713505

Clustering of genotypes between the control (top panel) and DIASTRAT (bottom panel) cohorts shown graphically for rs4713505

Gene	dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value		% AA			% AB			% BB		
				Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT	Control	DIASTRAT	*p*-value	Control	DIASTRAT	*p*-value	Control	DIASTRAT	*p*-value
KCNJ11	rs7841649	T	C	100	98.81	0.0789	0.0121	0.7087	0.8933	0.00	0.00	1.00	15.79	2.39	0.01	84.21	96.42	0.06
	rs76557252	T	G	100	100	0.0789	0.0045	0.7087	0.9626	0.00	0.00	1.00	15.79	0.89	0.00	84.21	99.11	0.00
	rs78374474	T	C	100	100	0.0789	0.0179	0.7087	0.4899	0.00	0.30	1.00	15.79	2.99	0.03	84.21	96.71	0.04
TCF7L2	rs4713505	T	G	100.00	100.00	0.3684	0.2015	0.0110	0.6215	0.00	4.77	0.524	73.68	30.75	0.000	26.32	64.48	0.002

Table 6.11 Difference in prevalence of SNPs in a control and diabetic cohort

A summary of SNPs that are significantly different between the non-diabetic control and the diabetic DIASTRAT cohort

6.3.3 Association of variants with glycaemic control

A major aim of the current study was to identify genetic markers of glycaemic control, and in particular, markers of glycaemic control in response to sulphonylurea treatment. The DIASTRAT cohort was therefore subdivided into those receiving sulphonylureas and those who were not. Subgroupings were further divided into lowest (Q1) and highest (Q4) quartiles according to HbA1c. In the group receiving sulphonylureas, this resulted in 21 individuals in Q1 and a further 17 individuals in Q4. In the non-sulphonylurea group, the numbers were 46 in Q1 and 32 in Q4.

Consistent with the analysis above, SNP call rates did not differ significantly between Q1 and Q4 in the sulphonylurea group (Tables 6.12 – 6.16). MAF scores again suggested that the vast majority of variants were common in the population (MAF >0.05).

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Q1	Q4	Q1	Q4	Q1	Q4
rs10961940	A	G	95.24	100.00	0.1250	0.1176	0.1599	0.5825
rs17743658	A	G	95.24	100.00	0.2500	0.3824	0.0369	0.1272
rs3738591	C	G	100.00	100.00	0.0476	0.0000	0.8188	1.0000
rs4741478	T	G	100.00	100.00	0.0952	0.0588	0.0404	0.7966
rs649224	A	G	100.00	100.00	0.0714	0.0588	0.7245	0.7966
rs9458011	T	C	100.00	100.00	0.0952	0.0882	0.6295	0.6899
rs8098346	T	G	100.00	100.00	0.4286	0.3235	0.3085	0.1762
rs75780075	A	G	100.00	100.00	0.0714	0.0588	0.7245	0.7966
rs73017157	A	G	100.00	100.00	0.0476	0.0000	0.8188	1.0000
rs11647027	A	C	100.00	100.00	0.0476	0.0000	0.8188	1.0000
rs59409558	A	C	95.24	100.00	0.0250	0.0000	0.9087	1.0000
rs11942387	A	G	100.00	100.00	0.3095	0.3824	0.0400	0.1272
rs62295555	T	C	100.00	100.00	0.0714	0.2059	0.7245	0.2851
rs12548842	T	C	100.00	100.00	0.4048	0.3529	0.1578	0.3487
rs79345026	T	C	100.00	100.00	0.1667	0.2059	0.5127	0.2851
rs10961999	T	C	100.00	100.00	0.2381	0.2059	0.0295	0.6785
rs111736238	T	G	100.00	100.00	0.0000	0.0294	1.0000	0.9006
rs114570814	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs116734533	T	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs78306404	T	C	95.24	100.00	0.0000	0.0000	1.0000	1.0000
rs111577081	T	C	100.00	100.00	0.0238	0.0588	0.9110	0.7966
rs117279260	T	C	100.00	100.00	0.0000	0.0294	1.0000	0.9006
rs117407497	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs2666826	T	C	100.00	100.00	0.2143	0.3235	0.9631	0.3877
rs2297775	T	C	100.00	100.00	0.3095	0.2059	0.3130	0.2851
rs10153859	A	G	100.00	100.00	0.0238	0.0294	0.9110	0.9006
rs17597643	A	G	100.00	100.00	0.0238	0.0294	0.9110	0.9006
rs58584712	A	G	100.00	100.00	0.1905	0.1765	0.0015	0.4322
rs78504459	T	C	100.00	100.00	0.0238	0.0000	0.9110	1.0000
rs150028933	-	A	100.00	100.00	0.0714	0.0000	0.0033	1.0000
rs200349598	A	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000

Table 6.12 Attributes of SNPs associated with ABCC8 for participants in the lowest (Q1) and highest (Q4) quartiles for HbA1c among those receiving sulphonylurea therapy.

31 unique SNPs associated with ABCC8 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* Value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Q1	Q4	Q1	Q4	Q1	Q4
rs1030986	T	C	100	100	0.0238	0.0000	0.9110	1.0000
rs16875779	A	G	100	100	0.1667	0.1765	0.3594	0.4322
rs17062197	A	G	100	100	0.1429	0.1471	0.4450	0.4772
rs2517646	T	C	100	100	0.2619	0.3235	0.0783	0.3877
rs2607614	T	C	100	100	0.0238	0.0000	0.9110	1.0000
rs33949518	T	C	95.24	100	0.2250	0.2941	0.9872	0.5363
rs34505188	A	G	100	100	0.0714	0.0294	0.7245	0.9006
rs35978505	T	C	100	100	0.0000	0.0588	1.0000	0.7966
rs369738	T	G	100	100	0.0476	0.0000	0.8188	1.0000
rs3815082	A	G	100	100	0.3571	0.2353	0.2091	0.2046
rs2849089	T	C	95.24	100	0.0000	0.0000	1.0000	1.0000
rs4479449	A	G	100	100	0.0714	0.1471	0.7245	0.4772
rs454748	A	G	100	100	0.2619	0.5000	0.5276	0.4669
rs683687	A	G	100	100	0.0714	0.1176	0.0033	0.5825
rs683866	T	G	100	100	0.4286	0.4706	0.0980	0.8188
rs6919617	A	G	100	100	0.0476	0.0882	0.8188	0.6899
rs7431770	A	G	100	100	0.0476	0.1176	0.8188	0.5825
rs757260	T	C	100	100	0.1905	0.1176	0.2809	0.5825
rs920829	T	C	100	100	0.1190	0.1765	0.5357	0.3770
rs9261151	A	G	100	100	0.0238	0.0294	0.9110	0.9006
rs9261216	A	G	100	100	0.0476	0.0882	0.8188	0.6899
rs9261302	A	G	100	100	0.0476	0.0882	0.8188	0.6899
rs9261471	T	C	100	100	0.3095	0.2059	0.3130	0.2851
rs9268135	A	G	95.24	94.12	0.3000	0.2500	0.2013	1.0000
rs9366752	T	C	100	100	0.1667	0.1471	0.0260	0.4772
rs9811423	C	G	100	100	0.4524	0.3824	0.7932	0.1198
rs1693703	T	C	100	100	0.1905	0.2941	0.0797	0.5363
rs7841649	T	C	95.24	100	0.0000	0.0294	1.0000	0.9006

Table 6.13 Attributes of SNPs associated with KCNJ11 for participants in the lowest (Q1) and highest (Q4) quartiles for HbA1c among those receiving sulphonylurea therapy.

101 unique SNPs associated with KCNJ11 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Q1	Q4	Q1	Q4	Q1	Q4
rs76592289	A	G	100	100	0.0714	0.0294	0.7245	0.9006
rs76557252	T	G	100	100	0.0000	0.0000	1.0000	1.0000
rs506757	T	G	100	100	0.3810	0.4412	0.0581	0.0962
rs62166939	A	C	95.24	100	0.2250	0.1765	0.1942	0.3770
rs78082945	T	C	100	100	0.0476	0.0588	0.8188	0.7966
rs62272114	A	G	100	100	0.0238	0.0000	0.9110	1.0000
rs112288451	T	C	100	100	0.0000	0.0294	1.0000	0.9006
rs74806059	T	C	100	100	0.0000	0.0000	1.0000	1.0000
rs79876945	A	G	100	100	0.0476	0.0588	0.8188	0.7966
rs114687906	A	C	100	100	0.0714	0.0000	0.7245	1.0000
rs115477611	T	C	100	100	0.0000	0.0000	1.0000	1.0000
rs78771971	T	C	100	100	0.0000	0.0000	1.0000	1.0000
rs78854530	A	G	100	100	0.0000	0.0294	1.0000	0.9006
rs62503295	T	C	100	100	0.0714	0.0294	0.7245	0.9006
rs114756881	T	C	95.24	82.35	0.0750	0.1429	0.7169	0.5329
rs4498896	T	C	100	100	0.4048	0.3529	0.1920	0.2352
rs79035182	T	G	100	100	0.0000	0.0294	1.0000	0.9006
rs74898314	A	G	100	100	0.0238	0.0000	0.9110	1.0000
rs28445507	A	C	100	100	0.2143	0.2647	0.2114	0.3135
rs55851518	A	G	100	100	0.0714	0.0588	0.7245	0.7966
rs79263726	A	C	100	100	0.0000	0.0294	1.0000	0.9006
rs10898365	T	G	100	100	0.2619	0.2353	0.5276	0.9368
rs66546253	A	G	100	94.12	0.1429	0.2188	0.3085	0.0710
rs783307	T	C	100	100	0.1190	0.1176	0.5357	0.5825
rs17734780	T	C	100	100	0.0000	0.0000	1.0000	1.0000
rs10432095	A	C	100	100	0.1190	0.0882	0.5357	0.6899
rs75107770	A	G	100	100	0.0000	0.0882	1.0000	0.6899
rs13061963	T	G	100	100	0.0952	0.0294	0.6295	0.9006

Table 6.13 Attributes of SNPs associated with KCNJ11 for participants in the lowest (Q1) and highest (Q4) quartiles for HbA1c among those receiving sulphonylurea therapy (continued)

101 unique SNPs associated with KCNJ11 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Q1	Q4	Q1	Q4	Q1	Q4
rs60400172	T	G	100	100	0.0714	0.0882	0.0033	0.0089
rs76451747	A	C	100	100	0.0000	0.0000	1.0000	1.0000
rs12520495	A	G	100	100	0.3095	0.2353	0.9903	0.2046
rs13183249	A	G	100	100	0.1905	0.2941	0.0797	0.0858
rs72725509	T	C	100	100	0.0000	0.0294	1.0000	0.9006
rs80184435	A	G	100	100	0.0238	0.0588	0.9110	0.7966
rs62404592	A	C	100	94.12	0.0238	0.0000	0.9110	1.0000
rs117910348	A	C	100	100	0.0000	0.0294	1.0000	0.9006
rs79853069	A	G	95.24	100	0.0750	0.0882	0.7169	0.6899
rs71521249	A	G	100	100	0.0238	0.0000	0.9110	1.0000
rs114234140	A	G	100	100	0.0000	0.0000	1.0000	1.0000
rs114249268	A	G	100	100	0.0476	0.0588	0.8188	0.0000
rs112623189	A	G	100	100	0.0238	0.0882	0.9110	0.6899
rs115010323	T	C	100	100	0.0000	0.0294	1.0000	0.9006
rs116250326	T	C	100	100	0.0000	0.0000	1.0000	1.0000
rs77111308	T	C	100	100	0.0000	0.0588	1.0000	0.7966
rs77823977	T	C	100	100	0.0476	0.0000	0.8188	1.0000
rs78374474	T	C	100	100	0.0238	0.0000	0.9110	1.0000
rs192657690	T	C	100	100	0.0238	0.0000	0.9110	1.0000
rs116983235	T	C	100	100	0.0238	0.0000	0.9110	1.0000
rs10846754	A	G	100	100	0.1429	0.2353	0.3085	0.2046
rs576184	A	C	100	100	0.4048	0.4706	0.6123	0.8188
rs16951460	A	G	100	100	0.0238	0.0000	0.9110	1.0000
rs3748592	A	G	100	100	0.0714	0.0588	0.7245	0.7966
rs2096376	T	C	100	100	0.4762	0.2353	0.2787	0.1535
rs32555	A	G	100	100	0.1905	0.1471	0.7362	0.4772
rs9261089	T	C	100	100	0.0238	0.0294	0.9110	0.9006

Table 6.13 Attributes of SNPs associated with KCNJ11 for participants in the lowest (Q1) and highest (Q4) quartiles for HbA1c among those receiving sulphonylurea therapy (continued)

101 unique SNPs associated with KCNJ11 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Q1	Q4	Q1	Q4	Q1	Q4
rs28400886	A	G	100	100	0.0476	0.0000	0.8188	1.0000
rs4959042	T	C	100	100	0.0000	0.0000	1.0000	1.0000
rs34704616	A	G	100	100	0.0000	0.0000	1.0000	1.0000
rs1573298	C	G	100	100	0.3333	0.2353	0.1017	0.2046
rs397596	T	C	100	100	0.0000	0.0000	1.0000	1.0000
rs139080520	A	G	100	100	0.0000	0.0000	1.0000	1.0000
rs13268757	A	G	100	100	0.1429	0.2647	0.4450	0.3135
rs114814001	T	C	100	100	0.0476	0.0294	0.8188	0.9006
rs79765628	T	C	100	100	0.0000	0.0000	1.0000	1.0000
rs117389196	T	C	100	100	0.0476	0.0000	0.8188	1.0000
rs141834826	A	G	100	100	0.0000	0.0000	1.0000	1.0000
rs201447432	T	C	100	100	0.0238	0.0000	0.9110	1.0000
rs146548891	T	G	100	100	0.0000	0.0000	1.0000	1.0000
rs142720326	A	G	100	100	0.0238	0.0000	0.9110	1.0000
rs201960928	A	G	100	100	0.0000	0.0000	1.0000	1.0000
rs139889337	A	G	100	100	0.0238	0.0000	0.9110	1.0000
rs113993403	T	C	100	100	0.0238	0.0294	0.9110	0.9006
rs138962514	T	C	100	100	0.0000	0.0000	1.0000	1.0000

Table 6.13 Attributes of SNPs associated with KCNJ11 for participants in the lowest (Q1) and highest (Q4) quartiles for HbA1c among those receiving sulphonylurea therapy (final)

101 unique SNPs associated with KCNJ11 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Q1	Q4	Q1	Q4	Q1	Q4
rs16841998	T	C	90.48	88.24	0.0263	0.1667	0.9062	0.4386
rs17461843	T	C	95.24	100.00	0.1000	0.0000	0.6193	1.0000
rs17571372	T	C	100	100.00	0.0714	0.0588	0.0033	0.7966
	T	G	100	100.00	0.0000	0.0000	1.0000	1.0000
rs3006968	A	G	100	100.00	0.5000	0.4412	0.5127	0.4965
rs6039591	A	G	100	100.00	0.0000	0.0588	1.0000	0.7966
rs6883010	A	G	100	100.00	0.4524	0.4118	0.7932	0.9062
rs6133707	A	G	100	100.00	0.2619	0.1471	0.6190	0.4772
rs77707280	A	G	100	100.00	0.0952	0.0294	0.0404	0.9006
rs807936	T	C	100	100.00	0.4762	0.4118	0.2787	0.0595
rs114348392	T	G	100	100.00	0.0238	0.0000	0.9110	1.0000
rs9356928	A	G	100	100.00	0.4048	0.4412	0.1578	0.7613
rs9460973	A	T	100	100.00	0.0238	0.0588	0.9110	0.7966
rs58554303	T	C	100	100.00	0.0476	0.0294	0.8188	0.9006
rs920628	A	G	100	100.00	0.0476	0.0882	0.8188	0.0089
rs16873732	T	C	100	100.00	0.0476	0.0882	0.8188	0.0089
rs1041791	T	C	100	100.00	0.1429	0.1765	0.4450	0.3770
rs61741363	A	G	100	100.00	0.0000	0.0000	1.0000	1.0000
rs113319303	A	G	100	100.00	0.0238	0.0588	0.9110	0.7966
rs116581086	A	G	100	100.00	0.0238	0.0000	0.9110	1.0000
rs74745471	A	G	100	100.00	0.0476	0.0294	0.8188	0.9006
rs61936141	T	C	100	100.00	0.0000	0.0294	1.0000	0.9006
rs79090092	A	G	100	100.00	0.0238	0.0294	0.9110	0.9006
rs10905750	A	C	100	100.00	0.0238	0.0294	0.9110	0.9006
rs231847	A	G	100	100.00	0.4048	0.3824	0.6899	0.1272
rs129072	T	C	95.24	100.00	0.1000	0.1765	0.0469	0.3770
rs75898263	T	C	100	100.00	0.0000	0.0000	1.0000	1.0000
rs111871569	A	G	95.24	100.00	0.0500	0.0000	0.8139	1.0000

Table 6.14 Attributes of SNPs associated with KCNQ1 for participants in the lowest (Q1) and highest (Q4) quartiles for HbA1c among those receiving sulphonylurea therapy.

68 unique SNPs associated with KCNQ1 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Q1	Q4	Q1	Q4	Q1	Q4
rs75875515	A	G	100	100.00	0.0000	0.0000	1.0000	1.0000
rs7156987	T	C	95.24	100.00	0.0000	0.0588	1.0000	0.7966
rs55767542	T	C	100	100.00	0.0238	0.0000	0.9110	1.0000
rs35162844	A	G	100	100.00	0.0476	0.0000	0.8188	1.0000
rs73063633	T	G	100	100.00	0.0476	0.0294	0.8188	0.9006
rs116045044	T	G	100	100.00	0.0714	0.0588	0.7245	0.7966
rs77692939	A	G	95.24	100.00	0.0000	0.0294	1.0000	0.9006
rs72811968	T	C	100	100.00	0.0238	0.0588	0.9110	0.7966
rs76238538	T	C	100	100.00	0.0238	0.0882	0.9110	0.6899
rs79931282	A	G	100	100.00	0.0476	0.0000	0.8188	1.0000
rs9467059	A	G	100	100.00	0.0238	0.0588	0.9110	0.7966
rs188539852	T	C	100	100.00	0.0000	0.0000	1.0000	1.0000
rs115704940	T	C	100	100.00	0.0238	0.0000	0.9110	1.0000
rs116858972	T	C	100	100.00	0.0238	0.0588	0.9110	0.7966
rs117103115	A	G	90.48	94.12	0.0526	0.0625	0.8087	0.7897
rs77936291	A	G	100	100.00	0.0238	0.0294	0.9110	0.9006
rs79947987	T	C	100	100.00	0.0000	0.0000	1.0000	1.0000
rs78468689	A	G	100	100.00	0.0714	0.0000	0.7245	1.0000
rs80140016	T	G	100	100.00	0.0476	0.0588	0.8188	0.7966
rs75894327	A	G	100	100.00	0.0238	0.0588	0.9110	0.7966
rs114351051	T	C	100	94.12	0.0952	0.0938	0.6295	0.6790
rs115005266	T	C	100	100.00	0.0476	0.0588	0.8188	0.7966
rs112214748	A	G	100	100.00	0.0714	0.0000	0.7245	1.0000
rs7119884	A	G	100	100.00	0.0476	0.0294	0.8188	0.9006
rs6057010	A	G	100	100.00	0.0476	0.0588	0.8188	0.7966
rs4690087	A	G	100	100.00	0.0238	0.0294	0.9110	0.9006
rs11754464	T	C	100	100.00	0.0000	0.0000	1.0000	1.0000
rs707938	A	G	100	94.12	0.3333	0.3125	0.5127	0.5128

Table 6.14 Attributes of SNPs associated with KCNQ1 for participants in the lowest (Q1) and highest (Q4) quartiles for HbA1c among those receiving sulphonylurea therapy (continued)

68 unique SNPs associated with KCNQ1 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Q1	Q4	Q1	Q4	Q1	Q4
rs35185125	A	C	100	100.00	0.0000	0.0000	1.0000	1.0000
rs28381355	A	G	100	100.00	0.0000	0.0000	1.0000	1.0000
rs183314661	A	C	100	100.00	0.0238	0.0294	0.9110	0.9006
rs77320475	A	G	100	100.00	0.0000	0.0882	1.0000	0.6899
rs17812699	A	G	100	100.00	0.1429	0.0588	0.4450	0.7966
rs199853687	A	C	100	100.00	0.0000	0.0000	1.0000	1.0000
rs115491500	T	C	100	100.00	0.0000	0.0000	1.0000	1.0000
rs11553182	T	C	95.24	100.00	0.1000	0.0294	0.6193	0.9006
	C	G	100	100.00	0.0000	0.0000	1.0000	1.0000
rs341047	A	G	100	100.00	0.0952	0.0588	0.6295	0.7966
rs150934987	A	G	100	100.00	0.0000	0.0000	1.0000	1.0000
rs139577182	A	G	100	100.00	0.0000	0.0000	1.0000	1.0000

Table 6.14 Attributes of SNPs associated with KCNQ1 for participants in the lowest (Q1) and highest (Q4) quartiles for HbA1c among those receiving sulphonylurea therapy (final)

68 unique SNPs associated with KCNQ1 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Q1	Q4	Q1	Q4	Q1	Q4
rs11755881	A	G	100.00	100.00	0.0714	0.0588	0.7245	0.7966
rs1861	A	C	100.00	100.00	0.0238	0.0294	0.9110	0.9006
rs28568667	T	C	90.48	88.24	0.0789	0.1333	0.7087	0.5513
rs4770748	A	G	100.00	100.00	0.0476	0.0294	0.8188	0.9006
rs663824	A	G	100.00	100.00	0.3333	0.3824	0.5127	0.0107
rs6905159	A	G	100.00	100.00	0.4762	0.3824	0.1232	0.1272
rs888576	T	C	100.00	100.00	0.1667	0.1176	0.3594	0.5825
rs13325508	T	C	100.00	100.00	0.0000	0.0294	1.0000	0.9006
rs55723035	T	C	100.00	100.00	0.0000	0.0588	1.0000	0.7966
rs77356515	T	G	100.00	100.00	0.0000	0.0294	1.0000	0.9006
rs75363174	A	G	100.00	100.00	0.0238	0.0882	0.9110	0.6899
rs4751674	T	C	100.00	100.00	0.3810	0.3529	0.9649	0.3487
rs61433965	A	G	100.00	100.00	0.0714	0.0000	0.7245	1.0000
rs58838391	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs76280424	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs77794667	T	C	100.00	100.00	0.0238	0.0000	0.9110	1.0000
rs59559871	T	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs72826927	A	G	100.00	100.00	0.0714	0.0882	0.7245	0.6899
rs945094	A	T	100.00	100.00	0.0238	0.0000	0.9110	1.0000
rs72751116	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs59706474	T	C	100.00	100.00	0.0238	0.0294	0.9110	0.9006
rs73251982	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs72489176	A	G	100.00	100.00	0.1667	0.2353	0.0260	0.1535
rs77367389	A	C	95.24	100.00	0.0250	0.0000	0.9087	1.0000
rs73668591	T	C	100.00	100.00	0.0000	0.0294	1.0000	0.9006
rs73669423	T	C	100.00	100.00	0.0952	0.0588	0.6295	0.7966
rs62493228	A	C	100.00	100.00	0.0714	0.0294	0.7245	0.9006
rs76569571	T	G	95.24	100.00	0.0750	0.0000	0.7169	1.0000

Table 6.15 Attributes of SNPs associated with HNF1α for participants in the lowest (Q1) and highest (Q4) quartiles for HbA1c among those receiving sulphonylurea therapy.

38 unique SNPs associated with HNF1α were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Q1	Q4	Q1	Q4	Q1	Q4
rs80312260	A	G	100.00	100.00	0.0000	0.0294	1.0000	0.9006
rs2244608	A	G	100.00	100.00	0.1667	0.4118	0.3594	0.3770
rs11616995	A	G	100.00	100.00	0.0476	0.0000	0.8188	1.0000
rs11647932	T	C	100.00	100.00	0.0476	0.1471	0.8188	0.4772
rs8102561	T	C	100.00	100.00	0.3571	0.1176	0.5190	0.5825
rs340141	T	C	100.00	100.00	0.2857	0.4412	0.0668	0.1979
rs150333766	T	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs189023122	T	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs150428096	T	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs147799118	T	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000

Table 6.15 Attributes of SNPs associated with HNF1α for participants in the lowest (Q1) and highest (Q4) quartiles for HbA1c among those receiving sulphonylurea therapy (final)

38 unique SNPs associated with HNF1α were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Q1	Q4	Q1	Q4	Q1	Q4
rs12894204	A	G	100.00	100.00	0.1190	0.2059	0.5357	0.6785
rs17605420	A	G	95.24	100.00	0.0250	0.0000	0.9087	1.0000
rs17763253	T	C	100.00	100.00	0.0714	0.0294	0.7245	0.9006
rs6728493	A	C	100.00	100.00	0.3095	0.2059	0.0400	0.6785
rs9906935	A	G	100.00	100.00	0.2619	0.2941	0.5276	0.5363
rs7089262	T	C	100.00	100.00	0.0714	0.0588	0.7245	0.7966
rs76677854	T	C	100.00	100.00	0.0714	0.0294	0.7245	0.9006
rs145079521	A	C	100.00	100.00	0.0476	0.0000	0.8188	1.0000
rs78082945	T	C	100.00	100.00	0.0476	0.0588	0.8188	0.7966
rs62272114	A	G	100.00	100.00	0.0238	0.0000	0.9110	1.0000
rs77385406	A	G	100.00	100.00	0.0000	0.0882	1.0000	0.6899
rs61732118	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs61875109	A	C	100.00	100.00	0.2619	0.2647	0.0783	0.8117
rs116369954	T	C	100.00	100.00	0.0952	0.0588	0.6295	0.7966
rs79805154	A	G	100.00	100.00	0.0714	0.0588	0.7245	0.7966
rs77064952	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs10059523	A	G	100.00	100.00	0.0714	0.0294	0.7245	0.9006
rs78183526	T	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs587667473	A	C	95.24	100.00	0.1750	0.1471	0.3428	0.4772
rs73179006	T	G	100.00	100.00	0.0714	0.0294	0.7245	0.9006
rs113262704	T	G	100.00	100.00	0.0952	0.0588	0.6295	0.7966
rs61989114	A	G	100.00	100.00	0.0000	0.0294	1.0000	0.9006
rs55656505	T	C	100.00	100.00	0.0476	0.0588	0.8188	0.7966
rs4783943	A	G	100.00	100.00	0.3333	0.2941	0.5127	0.0858
rs3026084	A	G	100.00	100.00	0.0476	0.0294	0.8188	0.9006
rs189314829	A	G	100.00	100.00	0.0476	0.0588	0.8188	0.7966
rs75107770	A	G	100.00	100.00	0.0000	0.0882	1.0000	0.6899
rs73223028	T	C	100.00	100.00	0.0000	0.0294	1.0000	0.9006

Table 6.16 Attributes of SNPs associated with TCF7L2 for participants in the lowest (Q1) and highest (Q4) quartiles for HbA1c among those receiving sulphonylurea therapy.

44 unique SNPs associated with TCF7L2 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value	
			Q1	Q4	Q1	Q4	Q1	Q4
rs116929578	A	G	95.24	94.12	0.0500	0.0938	0.8139	0.6790
rs117041486	A	G	100.00	100.00	0.0000	0.0294	1.0000	0.9006
rs79565172	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs117582734	T	C	100.00	100.00	0.0714	0.0294	0.7245	0.9006
rs12243326	T	C	100.00	100.00	0.2857	0.5000	0.7600	0.8084
rs4889830	A	G	100.00	100.00	0.4048	0.5000	0.6899	0.0896
rs3787493	A	G	100.00	100.00	0.4762	0.3824	0.2787	0.5971
rs4713505	T	G	100.00	100.00	0.2857	0.2647	0.7600	0.8117
rs2070600	T	C	100.00	100.00	0.0952	0.1176	0.6295	0.5825
rs3131300	A	G	100.00	100.00	0.2381	0.2941	0.3302	0.0740
rs73471190	T	C	100.00	100.00	0.0000	0.0294	1.0000	0.9006
rs148924158	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs143828311	T	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs201967398	A	G	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs143652701	T	C	100.00	100.00	0.0000	0.0000	1.0000	1.0000
rs142236510	T	C	100.00	100.00	0.0000	0.0588	1.0000	0.7966

Table 6.16 Attributes of SNPs associated with TCF7L2 for participants in the lowest (Q1) and highest (Q4) quartiles for HbA1c among those receiving sulphonylurea therapy (final)

44 unique SNPs associated with TCF7L2 were identified. SNP call rate is given as a percentage of total participants. MAF, Minor Allele Frequency; H.W. P value, Hardy-Weinberg *P* value.

Within the sulphonylurea group, the AB genotype of rs17743658 associated with ABCC8 was observed at a significantly higher rate in Q4 (19.05% Q1 versus 64.71% Q4, *P*=0.011, 95% CI -0.792, -0.121, OR 0.0128; Table 6.17). However, this trend was not observed in the non-sulphonylurea group (39.15% Q1 versus 50.0% Q4, *P*=0.471) suggesting that this variant may have utility in prediction of good glycaemic control in those receiving sulphonylurea therapy. However, this clearly requires further validation. According to data available from the T2D Knowledge portal this SNP is significantly associated with height and although not significant, the direction of effect in relation to HbA1c was positive (i.e. associated with higher HbA1c).

Within the sulphonylurea group, the AB genotype of rs506757 associated with KCNJ11 was observed at a significantly higher rate in Q1 (66.67% Q1 versus 29.41% Q4, *P*=0.050, 95% CI 0.023, 0.722, OR 4.80; Table 6.18). This was not replicated in the non-sulphonylurea group (52.13% Q1 versus 37.50% Q4, *P*=0.239). Data from the T2D knowledge portal suggest an association of rs506757 with systolic blood pressure. Staying with SNPs associated with KCNJ11, the AB genotype of rs13183249 was more prevalent in Q4 (19.05% Q1 versus 58.82% Q4, *P*=0.029, 95% CI -0.739, -0.057, OR 0.165, Table 6.18). The AB genotype of rs13183249 was not observed in the non-sulphonylurea group where only the AB genotype was observed.

The AB genotype of rs663824, which is associated with HNF1α was more commonly observed in Q4 of the sulphonylurea group (38.10% Q1 versus 76.47% Q4, *P*=0.042, 95% CI -0.726, -0.041, OR 0.189 Table 6.20), this SNP is associated with BMI, triglycerides and bipolar disorder according to data from the T2D knowledge portal. The AA genotype of rs2244608 was more common in Q1 of the sulphonylurea group (66.67% Q1 versus 29.41% Q4, *P*=0.050, 95% CI 0.023, 0.722, OR 4.80; Table 6.20), this SNP is also significantly associated with a number of phenotype's according to the

T2D Knowledge portal including T2DM, HDL, LDL, Cholesterol, coronary artery disease, corrected insulin response, proinsulin, fasting insulin, two hour glucose, eGFR-cys, insulin at 30 min OGTT and the disposition index. Finally, within the sulphonylurea group, the AA genotype of rs8102561 was observed more frequently in Q4 (38.10% Q1 versus 76.47% Q4, *P*=0.042, 95% CI -0.726, -0.041, OR 0.189, Table 6.20), and after consulting the T2D knowledge portal was shown to be significantly associated with HOMA IR and modified Stumvoll insulin sensitivity index. There was no significant difference in the prevalence of any of these variants in the non-sulphonylurea group, again suggesting that these variants may have utility in the prediction of good glycaemic control for individuals receiving sulphonylureas.

Significant differences in the genotypes of those in Q1 and Q4 were not observed for any variant associated with either KCNQ1 (Table 6.19), or TCF7L2 (Table 6.21), However, all other significant differences are summarized together in Table 6.22.

dbSNP RS ID	Minor allele (A)	Major allele (B)	% AA						% AB						% BB					
			Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ra
rs10961940	A	G	76.19	76.47	1.000	-0.277	0.272	0.985	14.29	23.53	0.760	-0.397	0.212	0.542	4.76	0.00	1.000	-0.091	0.186	0.000
rs17743658	A	G	14.29	5.88	0.746	-0.156	0.324	2.667	19.05	64.71	0.011	-0.792	-0.121	0.128	61.90	29.41	0.076	-0.028	0.678	3.900
rs3738591	C	G	90.48	100.00	0.512	-0.274	0.084	0.000	9.52	0.00	0.512	-0.084	0.274	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs4741478	T	G	85.71	88.24	1.000	-0.265	0.214	0.800	9.52	11.76	1.000	-0.243	0.198	0.789	4.76	0.00	1.000	-0.091	0.186	0.000
rs649224	A	G	0.00	0.00	1.000	0.000	0.000	0.000	14.29	11.76	1.000	-0.214	0.265	1.250	85.71	88.24	1.000	-0.265	0.214	0.800
rs9458011	T	C	0.00	0.00	1.000	0.000	0.000	0.000	19.05	17.65	1.000	-0.247	0.275	1.098	80.95	82.35	1.000	-0.275	0.247	0.911
rs8098346	T	G	38.10	17.65	0.282	-0.124	0.533	2.872	38.10	29.41	0.826	-0.266	0.440	1.477	23.81	52.94	0.119	-0.644	0.061	0.278
rs75780075	A	G	85.71	88.24	1.000	-0.265	0.214	0.800	14.29	11.76	1.000	-0.214	0.265	1.250	0.00	0.00	1.000	0.000	0.000	0.000
rs73017157	A	G	0.00	0.00	1.000	0.000	0.000	0.000	9.52	0.00	0.512	-0.084	0.274	0.000	90.48	100.00	0.542	-0.274	0.084	0.000
rs11647027	A	C	90.48	100.00	0.512	-0.274	0.084	0.000	9.52	0.00	0.512	-0.084	0.274	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs59409558	A	C	90.48	100.00	0.512	-0.274	0.084	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs11942387	A	G	0.00	5.88	0.922	-0.224	0.106	0.000	61.90	64.71	1.000	-0.364	0.308	0.886	38.10	29.41	0.826	-0.266	0.440	1.477
rs62295555	T	C	0.00	0.00	1.000	0.000	0.000	0.000	14.29	41.18	0.128	-0.600	0.062	0.238	85.71	58.82	0.128	-0.062	0.600	4.200
rs12548842	T	C	23.81	17.65	0.949	-0.249	0.372	1.458	33.33	35.29	1.000	-0.343	0.304	0.917	42.86	47.06	1.000	-0.402	0.318	0.844
rs79345026	T	C	4.76	0.00	1.000	-0.091	0.186	0.000	23.81	41.18	0.426	-0.523	0.176	0.446	71.43	58.82	0.638	-0.231	0.483	1.750
rs10961999	T	C	14.29	5.88	0.746	-0.156	0.324	2.667	19.05	29.41	0.719	-0.431	0.224	0.565	66.67	64.71	1.000	-0.304	0.343	1.091
rs111736238	T	G	100.00	94.12	0.922	-0.106	0.224	0.000	0.00	5.88	0.922	-0.224	0.106	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs114570814	A	G	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs116734533	T	C	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs78306404	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	95.24	100.00	1.000	-0.186	0.091	0.000
rs111577081	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	11.76	0.854	-0.301	0.161	0.375	95.24	88.24	0.854	-0.161	0.301	2.667
rs117279260	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.922	-0.224	0.106	0.000	100.00	94.12	0.922	-0.106	0.224	0.000
rs117407497	A	G	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs2666826	T	C	61.90	41.18	0.335	-0.159	0.573	2.321	33.33	52.94	0.368	-0.561	0.169	0.444	4.76	5.88	1.000	-0.167	0.144	0.800
rs2297775	T	C	52.38	58.82	0.945	-0.434	0.306	0.770	33.33	41.18	0.873	-0.440	0.284	0.714	14.29	0.00	0.240	-0.060	0.346	0.000
rs10153859	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	5.88	1.000	-0.167	0.144	0.800	95.24	94.12	1.000	-0.144	0.167	1.250
rs17597643	A	G	95.24	94.12	1.000	-0.144	0.167	1.250	4.76	5.88	1.000	-0.167	0.144	0.800	0.00	0.00	1.000	0.000	0.000	0.000
rs58584712	A	G	14.29	5.88	0.746	-0.156	0.324	2.667	9.52	23.53	0.473	-0.431	0.151	0.342	76.19	70.59	0.985	-0.280	0.392	1.333
rs78504459	T	C	95.24	100.00	1.000	-0.186	0.091	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs150028933	-	A	90.48	100.00	0.512	-0.274	0.084	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	4.76	0.00	1.000	-0.091	0.186	0.000
rs200349598	A	C	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000

Table 6.17 SNPs associated with ABCC8

31 unique SNPs associated with ABCC8 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$.

dbSNP RS ID	Minor allele (A)	Major allele (B)	% AA						% AB						% BB					
			Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio
rs1030986	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	0.158	-0.186	0.091	0.000
rs16875779	A	G	0.00	5.88	0.573	-0.224	0.106	0.000	33.33	23.53	0.494	-0.240	0.436	1.625	66.67	70.59	0.920	-0.374	0.296	0.833
rs17062197	A	G	0.00	0.00	1.000	0.000	0.000	0.000	28.57	29.41	1.000	-0.307	0.290	0.960	71.43	70.59	1.000	-0.290	0.307	1.042
rs2517646	T	C	61.90	41.18	0.128	-0.159	0.573	2.321	23.81	52.94	0.130	-0.644	0.061	0.278	14.29	5.88	0.340	-0.156	0.324	2.667
rs2607614	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	0.158	-0.186	0.091	0.000
rs33949518	T	C	4.76	11.76	0.579	-0.301	0.161	0.375	33.33	35.29	1.000	-0.343	0.304	0.917	57.14	52.94	0.908	-0.318	0.402	1.185
rs34505188	A	G	0.00	0.00	1.000	0.000	0.000	0.000	14.29	5.88	0.340	-0.156	0.324	2.667	85.71	94.12	0.340	-0.324	0.156	0.375
rs35978505	T	C	100.00	88.24	0.229	-0.089	0.324	0.000	0.00	11.76	0.229	-0.324	0.089	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs369738	T	G	90.48	100.00	0.530	-0.274	0.084	0.000	9.52	0.00	0.564	-0.084	0.274	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs3815082	A	G	47.62	52.94	0.834	-0.426	0.319	0.808	33.33	47.06	0.358	-0.502	0.227	0.562	19.05	0.00	0.308	-0.031	0.412	0.000
rs2849089	T	C	95.24	100.00	0.158	-0.186	0.091	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs4479449	A	G	85.71	70.59	0.252	-0.165	0.468	2.500	14.29	29.41	0.252	-0.468	0.165	0.400	0.00	0.00	1.000	0.000	0.000	0.000
rs454748	A	G	9.52	29.41	0.110	-0.502	0.105	0.253	33.33	41.18	0.667	-0.440	0.284	0.714	57.14	29.41	0.076	-0.079	0.633	3.200
rs683687	A	G	4.76	0.00	1.000	-0.091	0.186	0.000	4.76	23.53	0.107	-0.462	0.087	0.163	90.48	76.47	0.261	-0.151	0.431	2.923
rs683866	T	G	23.81	23.53	1.000	-0.272	0.277	1.016	66.67	47.06	0.158	-0.169	0.561	2.250	9.52	29.41	0.110	-0.502	0.105	0.253
rs6919617	A	G	90.48	82.35	0.531	-0.192	0.355	2.036	9.52	17.65	0.554	-0.355	0.192	0.491	0.00	0.00	1.000	0.000	0.000	0.000
rs7431770	A	G	0.00	0.00	1.000	0.000	0.000	0.000	9.52	23.53	0.261	-0.431	0.151	0.342	90.48	76.47	0.261	-0.151	0.431	2.923
rs757260	T	C	0.00	0.00	1.000	0.000	0.000	0.000	38.10	23.53	0.251	-0.197	0.488	2.000	61.90	76.47	0.251	-0.488	0.197	0.500
rs920829	T	C	0.00	0.00	1.000	0.000	0.000	0.000	23.81	35.29	0.444	-0.459	0.230	0.573	76.19	64.71	0.444	-0.230	0.459	1.745
rs9261151	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	5.88	1.000	-0.167	0.144	0.800	95.24	94.12	1.000	-0.144	0.167	1.250
rs9261216	A	G	90.48	82.35	0.531	-0.192	0.355	2.036	9.52	17.65	0.554	-0.355	0.192	0.491	0.00	0.00	1.000	0.000	0.000	0.000
rs9261302	A	G	0.00	0.00	1.000	0.000	0.000	0.000	9.52	17.65	0.554	-0.355	0.192	0.491	90.48	82.35	0.554	-0.192	0.355	2.036
rs9261471	T	C	52.38	58.82	0.758	-0.434	0.306	0.770	33.33	41.18	0.667	-0.440	0.284	0.714	14.29	0.00	0.308	-0.060	0.346	0.000
rs9268135	A	G	52.38	52.94	1.000	-0.330	0.319	0.978	28.57	35.29	0.731	-0.419	0.284	0.733	14.29	5.88	0.340	-0.156	0.324	2.667
rs9366752	T	C	9.52	0.00	0.564	-0.084	0.274	0.000	14.29	29.41	0.252	-0.468	0.165	0.400	76.19	70.59	0.797	-0.280	0.392	1.333

Table 6.18 SNPs associated with KCNJ11

101 unique SNPs associated with KCNJ11 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$.

dbSNP RS ID	Minor allele (A)	Major allele (B)	% AA						% AB						% BB					
			Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio
rs9811423	C	G	28.57	47.06	0.186	-0.544	0.174	0.450	52.38	29.41	0.067	-0.128	0.587	2.640	19.05	23.53	0.869	-0.352	0.262	0.765
rs1693703	T	C	71.43	52.94	0.186	-0.174	0.544	2.222	19.05	35.29	0.234	-0.498	0.173	0.431	9.52	11.76	1.000	-0.243	0.198	0.789
rs7841649	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.573	-0.224	0.106	0.000	95.24	94.12	1.000	-0.144	0.167	1.250
rs76592289	A	G	0.00	0.00	1.000	0.000	0.000	0.000	14.29	5.88	0.340	-0.156	0.324	2.667	85.71	94.12	0.340	-0.324	0.156	0.375
rs76557252	T	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs506757	T	G	28.57	41.18	0.402	-0.483	0.231	0.571	66.67	29.41	0.050	0.023	0.722	4.800	4.76	29.41	0.104	-0.535	0.042	0.120
rs62166939	A	C	0.00	0.00	1.000	0.000	0.000	0.000	42.86	35.29	0.678	-0.288	0.439	1.375	52.38	64.71	0.406	-0.488	0.242	0.600
rs78082945	T	C	90.48	88.24	1.000	-0.198	0.243	1.267	9.52	11.76	1.000	-0.243	0.198	0.789	0.00	0.00	1.000	0.000	0.000	0.000
rs62272114	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	0.158	-0.186	0.091	0.000
rs112288451	T	C	100.00	94.12	0.573	-0.106	0.224	0.000	0.00	5.88	0.573	-0.224	0.106	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs74806059	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs79876945	A	G	0.00	0.00	1.000	0.000	0.000	0.000	9.52	11.76	1.000	-0.243	0.198	0.789	90.48	88.24	1.000	-0.198	0.243	1.267
rs114687906	A	C	0.00	0.00	1.000	0.000	0.000	0.000	14.29	0.00	0.308	-0.060	0.346	0.000	85.71	100.00	0.308	-0.346	0.060	0.000
rs115477611	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs78771971	T	C	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs78854530	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.573	-0.224	0.106	0.000	100.00	94.12	0.573	-0.106	0.224	0.000
rs62503295	T	C	85.71	94.12	0.340	-0.324	0.156	0.375	14.29	5.88	0.340	-0.156	0.324	2.667	0.00	0.00	1.000	0.000	0.000	0.000
rs114756881	T	C	80.95	58.82	0.097	-0.120	0.563	2.975	14.29	23.53	0.521	-0.397	0.212	0.542	0.00	0.00	1.000	0.000	0.000	0.000
rs4498896	T	C	9.52	5.88	0.887	-0.168	0.241	1.684	61.90	58.82	0.983	-0.313	0.374	1.137	28.57	35.29	0.731	-0.419	0.284	0.733
rs79035182	T	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.573	-0.224	0.106	0.000	100.00	94.12	0.573	-0.106	0.224	0.000
rs74898314	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	0.158	-0.186	0.091	0.000

Table 6.18 SNPs associated with KCNJ11 (continued)

101 unique SNPs associated with KCNJ11 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$.

dbSNP RS ID	Minor allele (A)	Major allele (B)	% AA						% AB						% BB					
			Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio
rs28445507	A	C	0.00	11.76	0.229	-0.324	0.089	0.000	42.86	29.41	0.331	-0.222	0.491	1.800	57.14	58.82	1.000	-0.349	0.315	0.933
rs55851518	A	G	0.00	0.00	1.000	0.000	0.000	0.000	14.29	11.76	1.000	-0.214	0.265	1.250	85.71	88.24	1.000	-0.265	0.214	0.800
rs79263726	A	C	100.00	94.12	0.573	-0.106	0.224	0.000	0.00	5.88	0.573	-0.224	0.106	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs10898365	T	G	9.52	5.88	0.890	-0.168	0.241	1.684	33.33	35.29	1.000	-0.343	0.304	0.917	57.14	58.82	1.000	-0.349	0.315	0.933
rs66546253	A	G	4.76	11.76	0.579	-0.301	0.161	0.375	19.05	17.65	1.000	-0.247	0.275	1.098	76.19	64.71	0.444	-0.230	0.459	1.745
rs783307	T	C	76.19	76.47	1.000	-0.277	0.272	0.985	23.81	23.53	1.000	-0.272	0.277	1.016	0.00	0.00	1.000	0.000	0.000	0.000
rs17734780	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs10432095	A	C	76.19	82.35	0.716	-0.372	0.249	0.686	23.81	17.65	0.716	-0.249	0.372	1.458	0.00	0.00	1.000	0.000	0.000	0.000
rs75107770	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	17.65	0.093	-0.411	0.058	0.000	100.00	82.35	0.093	-0.058	0.411	0.000
rs13061963	T	G	0.00	0.00	1.000	0.000	0.000	0.000	19.05	5.88	0.084	-0.123	0.387	3.765	80.95	94.12	0.084	-0.387	0.123	0.266
rs60400172	T	G	4.76	5.88	1.000	-0.167	0.144	0.800	4.76	5.88	1.000	-0.167	0.144	0.800	90.48	88.24	1.000	-0.198	0.243	1.267
rs76451747	A	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs12520495	A	G	47.62	52.94	0.834	-0.426	0.319	0.808	42.86	47.06	0.908	-0.402	0.318	0.844	9.52	0.00	0.564	-0.084	0.274	0.000
rs13183249	A	G	9.52	0.00	0.564	-0.084	0.274	0.000	19.05	58.82	0.029	-0.739	-0.057	0.165	71.43	41.18	0.126	-0.054	0.659	3.571
rs72725509	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.573	-0.224	0.106	0.000	100.00	94.12	0.573	-0.106	0.224	0.000
rs80184435	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	11.76	0.579	-0.301	0.161	0.375	95.24	88.24	0.579	-0.161	0.301	2.667
rs62404592	A	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	94.12	1.000	-0.144	0.167	1.250
rs117910348	A	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.573	-0.224	0.106	0.000	100.00	94.12	0.573	-0.106	0.224	0.000
rs79853069	A	G	80.95	82.35	1.000	-0.275	0.247	0.911	14.29	17.65	0.953	-0.302	0.235	0.778	0.00	0.00	1.000	0.000	0.000	0.000
rs71521249	A	G	95.24	100.00	0.158	-0.186	0.091	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs114234140	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs114249268	A	G	0.00	5.88	0.573	-0.224	0.106	0.000	9.52	0.00	0.564	-0.084	0.274	0.000	90.48	94.12	0.887	-0.241	0.168	0.594
rs112623189	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	17.65	0.257	-0.385	0.127	0.233	95.24	82.35	0.257	-0.127	0.385	4.286
rs115010323	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.573	-0.224	0.106	0.000	100.00	94.12	0.573	-0.106	0.224	0.000
rs116250326	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs77111308	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	11.76	0.229	-0.324	0.089	0.000	100.00	88.24	0.229	-0.089	0.324	0.000
rs77823977	T	C	90.48	100.00	0.564	-0.274	0.084	0.000	9.52	0.00	0.564	-0.084	0.274	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs78374474	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	0.158	-0.186	0.091	0.000
rs192657690	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	0.158	-0.186	0.091	0.000
rs116983235	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	0.158	-0.186	0.091	0.000

Table 6.18 SNPs associated with KCNJ11 (continued)

101 unique SNPs associated with KCNJ11 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$.

dbSNP RS ID	Minor allele (A)	Major allele (B)	% AA						% AB						% BB					
			Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio
rs10846754	A	G	4.76	0.00	1.000	-0.091	0.186	0.000	19.05	47.06	0.135	-0.624	0.064	0.265	76.19	52.94	0.085	-0.120	0.585	2.844
rs576184	A	C	19.05	23.53	0.869	-0.352	0.262	0.765	42.86	47.06	0.908	-0.402	0.318	0.844	38.10	29.41	0.591	-0.266	0.440	1.477
rs16951460	A	G	95.24	100.00	0.158	-0.186	0.091	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs3748592	A	G	0.00	0.00	1.000	0.000	0.000	0.000	14.29	11.76	1.000	-0.214	0.265	1.250	85.71	88.24	1.000	-0.265	0.214	0.800
rs2096376	T	C	33.33	64.71	0.115	-0.671	0.043	0.273	38.10	23.53	0.251	-0.197	0.488	2.000	28.57	11.76	0.080	-0.132	0.468	3.000
rs32555	A	G	66.67	70.59	0.920	-0.374	0.296	0.833	28.57	29.41	1.000	-0.307	0.290	0.960	4.76	0.00	0.158	-0.091	0.186	0.000
rs9261089	T	C	95.24	94.12	1.000	-0.144	0.167	1.250	4.76	5.88	1.000	-0.167	0.144	0.800	0.00	0.00	1.000	0.000	0.000	0.000
rs28400886	A	G	0.00	0.00	1.000	0.000	0.000	0.000	9.52	0.00	0.564	-0.084	0.274	0.000	90.48	100.00	0.564	-0.274	0.084	0.000
rs4959042	T	C	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs34704616	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs1573298	C	G	19.05	0.00	0.257	-0.031	0.412	0.000	28.57	47.06	0.186	-0.544	0.174	0.450	52.38	52.94	1.000	-0.330	0.319	0.978
rs397596	T	C	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs139080520	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs13268757	A	G	0.00	11.76	0.229	-0.324	0.089	0.000	28.57	29.41	1.000	-0.307	0.290	0.960	71.43	58.82	0.402	-0.231	0.483	1.750
rs114814001	T	C	0.00	0.00	1.000	0.000	0.000	0.000	9.52	5.88	0.887	-0.168	0.241	1.684	90.48	94.12	0.887	-0.241	0.168	0.594
rs79765628	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs117389196	T	C	0.00	0.00	1.000	0.000	0.000	0.000	9.52	0.00	0.564	-0.084	0.274	0.000	90.48	100.00	0.564	-0.274	0.084	0.000
rs141834826	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs201447432	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	0.158	-0.186	0.091	0.000
rs146548891	T	G	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs142720326	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	0.158	-0.186	0.091	0.000
rs201960928	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs139889337	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	0.158	-0.186	0.091	0.000
rs113993403	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	5.88	1.000	-0.167	0.144	0.800	95.24	94.12	1.000	-0.144	0.167	1.250
rs138962514	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000

Table 6.18 SNPs associated with KCNJ11 (final)

101 unique SNPs associated with KCNJ11 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$.

dbSNP RS ID	Minor allele (A)	Major allele (B)	% AA						% AB						% BB					
			Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio
rs16841998	T	C	85.71	58.82	0.133	-0.062	0.600	4.200	4.76	29.41	0.104	-0.535	0.042	0.120	0.00	0.00	1.000	0.000	0.000	0.000
rs17461843	T	C	76.19	100.00	0.094	-0.473	-0.003	0.000	19.05	0.00	0.170	-0.031	0.412	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs17571372	T	C	4.76	0.00	1.000	-0.091	0.186	0.000	4.76	11.76	0.849	-0.301	0.161	0.375	90.48	88.24	1.000	-0.198	0.243	1.267
	T	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs3006968	A	G	28.57	23.53	1.000	-0.279	0.380	1.300	42.86	41.18	1.000	-0.315	0.349	1.071	28.57	35.29	0.927	-0.419	0.284	0.733
rs6039591	A	G	100.00	88.24	0.377	-0.089	0.324	0.000	0.00	11.76	0.377	-0.324	0.089	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs6883010	A	G	19.05	17.65	1.000	-0.247	0.275	1.098	52.38	47.06	1.000	-0.319	0.426	1.238	28.57	35.29	0.927	-0.419	0.284	0.733
rs6133707	A	G	52.38	70.59	0.431	-0.540	0.175	0.458	42.86	29.41	0.606	-0.222	0.491	1.800	4.76	0.00	1.000	-0.091	0.186	0.000
rs77707280	A	G	4.76	0.00	1.000	-0.091	0.186	0.000	9.52	5.88	1.000	-0.168	0.241	1.684	85.71	94.12	0.758	-0.324	0.156	0.375
rs807936	T	C	28.57	23.53	1.000	-0.279	0.380	1.300	38.10	70.59	0.095	-0.678	0.028	0.256	33.33	5.88	0.096	-0.009	0.558	8.000
rs114348392	T	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	1.000	-0.186	0.091	0.000
rs9356928	A	G	23.81	29.41	0.985	-0.392	0.280	0.750	33.33	52.94	0.375	-0.561	0.169	0.444	42.86	17.65	0.190	-0.080	0.584	3.500
rs9460973	A	T	0.00	0.00	1.000	0.000	0.000	0.000	4.76	11.76	0.849	-0.301	0.161	0.375	95.24	88.24	0.849	-0.161	0.301	2.667
rs58554303	T	C	90.48	94.12	1.000	-0.241	0.168	0.594	9.52	5.88	1.000	-0.168	0.241	1.684	0.00	0.00	1.000	0.000	0.000	0.000
rs920628	A	G	90.48	88.24	1.000	-0.198	0.243	1.267	9.52	5.88	1.000	-0.168	0.241	1.684	0.00	5.88	0.915	-0.224	0.106	0.000
rs16873732	T	C	90.48	88.24	1.000	-0.198	0.243	1.267	9.52	5.88	1.000	-0.168	0.241	1.684	0.00	5.88	0.915	-0.224	0.106	0.000
rs1041791	T	C	71.43	64.71	0.927	-0.284	0.419	1.364	28.57	35.29	0.927	-0.419	0.284	0.733	0.00	0.00	1.000	0.000	0.000	0.000
rs61741363	A	G	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs113319303	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	11.76	0.849	-0.301	0.161	0.375	95.24	88.24	0.849	-0.161	0.301	2.667
rs116581086	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	1.000	-0.186	0.091	0.000
rs74745471	A	G	0.00	0.00	1.000	0.000	0.000	0.000	9.52	5.88	1.000	-0.168	0.241	1.684	90.48	94.12	1.000	-0.241	0.168	0.594
rs61936141	T	C	100.00	94.12	0.915	-0.106	0.224	0.000	0.00	5.88	0.915	-0.224	0.106	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs79090092	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	5.88	1.000	-0.167	0.144	0.800	95.24	94.12	1.000	-0.144	0.167	1.250
rs10905750	A	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	5.88	1.000	-0.167	0.144	0.800	95.24	94.12	1.000	-0.144	0.167	1.250
rs231847	A	G	14.29	5.88	0.746	-0.156	0.324	2.667	52.38	64.71	0.664	-0.488	0.242	0.600	33.33	29.41	1.000	-0.296	0.374	1.200

Table 6.19 SNPs associated with KCNQ1

68 unique SNPs associated with KCNQ1 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

dbSNP RS ID	Minor allele (A)	Major allele (B)	% AA						% AB						% BB					
			Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio
rs129072	T	C	4.76	0.00	1.000	-0.091	0.186	0.000	9.52	35.29	0.124	-0.570	0.055	0.193	80.95	64.71	0.447	-0.173	0.498	2.318
rs75898263	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs111871569	A	G	0.00	0.00	1.000	0.000	0.000	0.000	9.52	0.00	0.564	-0.084	0.274	0.000	85.71	100.00	0.308	-0.346	0.060	0.000
rs75875515	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs7156987	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	11.76	0.377	-0.324	0.089	0.000	95.24	88.24	0.849	-0.161	0.301	2.667
rs55767542	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	1.000	-0.186	0.091	0.000
rs35162844	A	G	0.00	0.00	1.000	0.000	0.000	0.000	9.52	0.00	0.564	-0.084	0.274	0.000	90.48	100.00	0.564	-0.274	0.084	0.000
rs73063633	T	G	90.48	94.12	1.000	-0.241	0.168	0.594	9.52	5.88	1.000	-0.168	0.241	1.684	0.00	0.00	1.000	0.000	0.000	0.000
rs116045044	T	G	85.71	88.24	1.000	-0.265	0.214	0.800	14.29	11.76	1.000	-0.214	0.265	1.250	0.00	0.00	1.000	0.000	0.000	0.000
rs77692939	A	G	95.24	94.12	1.000	-0.144	0.167	1.250	0.00	5.88	0.915	-0.224	0.106	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs72811968	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	11.76	0.849	-0.301	0.161	0.375	95.24	88.24	0.849	-0.161	0.301	2.667
rs76238538	T	C	95.24	82.35	0.450	-0.127	0.385	4.286	4.76	17.65	0.450	-0.385	0.127	0.233	0.00	0.00	1.000	0.000	0.000	0.000
rs79931282	A	G	90.48	100.00	0.564	-0.274	0.084	0.000	9.52	0.00	0.564	-0.084	0.274	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs9467059	A	G	95.24	88.24	0.849	-0.161	0.301	2.667	4.76	11.76	0.849	-0.301	0.161	0.375	0.00	0.00	1.000	0.000	0.000	0.000
rs188539852	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs115704940	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	1.000	-0.186	0.091	0.000
rs116858972	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	11.76	0.849	-0.301	0.161	0.375	95.24	88.24	0.849	-0.161	0.301	2.667
rs117103115	A	G	80.95	82.35	1.000	-0.275	0.247	0.911	9.52	11.76	1.000	-0.243	0.198	0.789	0.00	0.00	1.000	0.000	0.000	0.000
rs77936291	A	G	95.24	94.12	1.000	-0.144	0.167	1.250	4.76	5.88	1.000	-0.167	0.144	0.800	0.00	0.00	1.000	0.000	0.000	0.000
rs79947987	T	C	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs78468689	A	G	0.00	0.00	1.000	0.000	0.000	0.000	14.29	0.00	0.308	-0.060	0.346	0.000	85.71	100.00	0.308	-0.346	0.060	0.000
rs80140016	T	G	0.00	0.00	1.000	0.000	0.000	0.000	9.52	11.76	1.000	-0.243	0.198	0.789	90.48	88.24	1.000	-0.198	0.243	1.267
rs75894327	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	11.76	0.849	-0.301	0.161	0.375	95.24	88.24	0.849	-0.161	0.301	2.667
rs114351051	T	C	80.95	76.47	1.000	-0.262	0.352	1.308	19.05	17.65	1.000	-0.247	0.275	1.098	0.00	0.00	1.000	0.000	0.000	0.000
rs115005266	T	C	0.00	0.00	1.000	0.000	0.000	0.000	9.52	11.76	1.000	-0.243	0.198	0.789	90.48	88.24	1.000	-0.198	0.243	1.267
rs112214748	A	G	0.00	0.00	1.000	0.000	0.000	0.000	14.29	0.00	0.308	-0.060	0.346	0.000	85.71	100.00	0.308	-0.346	0.060	0.000

Table 6.19 SNPs associated with KCNQ1 (continued)

68 unique SNPs associated with KCNQ1 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

			% AA						% AB						% BB					
dbSNP RS ID	Minor allele (A)	Major allele (B)	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio
rs7119884	A	G	90.48	94.12	1.000	-0.241	0.168	0.594	9.52	5.88	1.000	-0.168	0.241	1.684	0.00	0.00	1.000	0.000	0.000	0.000
rs6057010	A	G	90.48	88.24	1.000	-0.198	0.243	1.267	9.52	11.76	1.000	-0.243	0.198	0.789	0.00	0.00	1.000	0.000	0.000	0.000
rs4690087	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	5.88	1.000	-0.167	0.144	0.800	95.24	94.12	1.000	-0.144	0.167	1.250
rs11754464	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs707938	A	G	47.62	41.18	0.945	-0.306	0.434	1.299	38.10	47.06	0.821	-0.458	0.279	0.692	14.29	5.88	0.758	-0.156	0.324	2.667
rs35185125	A	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs28381355	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs183314661	A	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	5.88	1.000	-0.167	0.144	0.800	95.24	94.12	1.000	-0.144	0.167	1.250
rs77320475	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	17.65	0.161	-0.411	0.058	0.000	100.00	82.35	0.308	-0.058	0.411	0.000
rs17812699	A	G	0.00	0.00	1.000	0.000	0.000	0.000	28.57	11.76	0.388	-0.132	0.468	3.000	71.43	88.24	0.388	-0.468	0.132	0.333
rs199853687	A	C	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs115491500	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs11553182	T	C	0.00	0.00	1.000	0.000	0.000	0.000	19.05	5.88	0.477	-0.123	0.387	3.765	76.19	94.12	0.388	-0.446	0.088	0.200
	C	G	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs341047	A	G	0.00	0.00	1.000	0.000	0.000	0.000	19.05	11.76	0.869	-0.208	0.353	1.765	80.95	88.24	0.388	-0.353	0.208	0.567
rs150934987	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	0.388	0.000	0.000	0.000
rs139577182	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000

Table 6.19 SNPs associated with KCNQ1 (final)

68 unique SNPs associated with KCNQ1 were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

dbSNP RS ID	Minor allele (A)	Major allele (B)	% AA						% AB						% BB					
			Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio
rs11755881	A	G	0.00	0.00	1.000	0.000	0.000	0.000	14.29	11.76	1.000	-0.214	0.265	1.250	85.71	88.24	1.000	-0.265	0.214	0.800
rs1861	A	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	5.88	0.746	-0.167	0.144	0.800	95.24	94.12	1.000	-0.144	0.167	1.250
rs28568667	T	C	76.19	64.71	0.677	-0.230	0.459	1.745	14.29	23.53	0.757	-0.397	0.212	0.542	0.00	0.00	1.000	0.000	0.000	0.000
rs4770748	A	G	0.00	0.00	1.000	0.000	0.000	0.000	9.52	5.88	1.000	-0.168	0.241	1.684	90.48	94.12	1.000	-0.241	0.168	0.594
rs663824	A	G	47.62	23.53	0.233	-0.106	0.588	2.955	38.10	76.47	0.042	-0.726	-0.041	0.189	14.29	0.00	0.240	-0.060	0.346	0.000
rs6905159	A	G	19.05	29.41	0.716	-0.431	0.224	0.565	66.67	64.71	1.000	-0.304	0.343	1.091	14.29	5.88	0.746	-0.156	0.324	2.667
rs888576	T	C	0.00	0.00	1.000	0.000	0.000	0.000	33.33	23.53	0.762	-0.240	0.436	1.625	66.67	76.47	0.762	-0.436	0.240	0.615
rs13325508	T	C	100.00	94.12	0.915	-0.106	0.224	0.000	0.00	5.88	0.922	-0.224	0.106	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs55723035	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	11.76	0.377	-0.324	0.089	0.000	100.00	88.24	0.377	-0.089	0.324	0.000
rs77356515	T	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.922	-0.224	0.106	0.000	100.00	94.12	0.915	-0.106	0.224	0.000
rs75363174	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	17.65	0.450	-0.385	0.127	0.233	95.24	82.35	0.450	-0.127	0.385	4.286
rs4751674	T	C	14.29	17.65	1.000	-0.302	0.235	0.778	47.62	35.29	0.664	-0.242	0.488	1.667	38.10	47.06	0.821	-0.458	0.279	0.692
rs61433965	A	G	85.71	100.00	0.308	-0.346	0.060	0.000	14.29	0.00	0.240	-0.060	0.346	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs58838391	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs76280424	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs77794667	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	1.000	-0.186	0.091	0.000
rs59559871	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs72826927	A	G	0.00	0.00	1.000	0.000	0.000	0.000	14.29	17.65	1.000	-0.302	0.235	0.778	85.71	82.35	1.000	-0.235	0.302	1.286
rs945094	A	T	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	1.000	-0.186	0.091	0.000
rs72751116	A	G	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs59706474	T	C	0.00	0.00	1.000	0.000	0.000	0.000	4.76	5.88	1.000	-0.167	0.144	0.800	95.24	94.12	1.000	-0.144	0.167	1.250
rs73251982	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs72489176	A	G	76.19	64.71	0.677	-0.230	0.459	1.745	14.29	23.53	0.757	-0.397	0.212	0.542	9.52	11.76	1.000	-0.243	0.198	0.789

Table 6.20 SNPs associated with HNF1α

38 unique SNPs associated with HNF1α were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

dbSNP RS ID	Minor allele (A)	Major allele (B)	% AA						% AB						% BB					
			Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio
rs77367389	A	C	90.48	100.00	0.564	-0.274	0.084	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs73668591	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.922	-0.224	0.106	0.000	100.00	94.12	0.915	-0.106	0.224	0.000
rs73669423	T	C	80.95	88.24	0.869	-0.353	0.208	0.567	19.05	11.76	0.869	-0.208	0.353	1.765	0.00	0.00	1.000	0.000	0.000	0.000
rs62493228	A	C	0.00	0.00	1.000	0.000	0.000	0.000	14.29	5.88	0.746	-0.156	0.324	2.667	85.71	94.12	0.915	-0.324	0.156	0.375
rs76569571	T	G	0.00	0.00	1.000	0.000	0.000	0.000	14.29	0.00	0.240	-0.060	0.346	0.000	80.95	100.00	0.170	-0.412	0.031	0.000
rs80312260	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.922	-0.224	0.106	0.000	100.00	94.12	0.915	-0.106	0.224	0.000
rs2244608	A	G	66.67	29.41	0.050	0.023	0.722	4.800	33.33	58.82	0.214	-0.617	0.107	0.350	0.00	11.76	0.377	-0.324	0.089	0.000
rs11616995	A	G	90.48	100.00	0.564	-0.274	0.084	0.000	9.52	0.00	0.512	-0.084	0.274	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs11647932	T	C	0.00	0.00	1.000	0.000	0.000	0.000	9.52	29.41	0.249	-0.502	0.105	0.253	90.48	70.59	0.249	-0.105	0.502	3.958
rs8102561	T	C	38.10	76.47	0.042	-0.726	-0.041	0.189	52.38	23.53	0.140	-0.058	0.635	3.575	9.52	0.00	0.512	-0.084	0.274	0.000
rs340141	T	C	0.00	23.53	0.069	-0.490	0.020	0.000	57.14	64.71	0.888	-0.439	0.288	0.727	42.86	11.76	0.081	-0.004	0.625	5.625
rs150333766	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs189023122	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs150428096	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs147799118	T	C	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000

Table 6.20 SNPs associated with HNF1α (final)

38 unique SNPs associated with HNF1α were identified, and genotyped. Following a two-sample t-test for proportions, significant differences in the prevalence of genotypes are highlighted in red. Significance was accepted at $P<0.05$

dbSNP RS ID	Minor allele (A)	Major allele (B)	% AA						% AB						% BB					
			Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio
rs12894204	A	G	0.00	5.88	0.922	-0.224	0.106	0.000	23.81	29.41	0.985	-0.392	0.280	0.750	76.19	64.71	0.679	-0.230	0.459	1.745
rs17605420	A	G	90.48	100.00	0.512	-0.274	0.084	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs17763253	T	C	0.00	0.00	1.000	0.000	0.000	0.000	14.29	5.88	0.746	-0.156	0.324	2.669	85.71	94.12	0.746	-0.324	0.156	0.375
rs6728493	A	C	38.10	64.71	0.175	-0.627	0.095	0.336	61.90	29.41	0.076	-0.028	0.678	3.900	0.00	5.88	0.922	-0.224	0.106	0.000
rs9906935	A	G	57.14	52.94	1.000	-0.318	0.402	1.185	33.33	35.29	1.000	-0.343	0.304	0.917	9.52	11.76	1.000	-0.243	0.198	0.789
rs7089262	T	C	0.00	0.00	1.000	0.000	0.000	0.000	14.29	11.76	1.000	-0.214	0.265	1.251	85.71	88.24	1.000	-0.265	0.214	0.799
rs76677854	T	C	85.71	94.12	0.746	-0.324	0.156	0.375	14.29	5.88	0.746	-0.156	0.324	2.669	0.00	0.00	1.000	0.000	0.000	0.000
rs145079521	A	C	90.48	100.00	0.512	-0.274	0.084	0.000	9.52	0.00	0.512	-0.084	0.274	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs78082945	T	C	90.48	88.24	1.000	-0.198	0.243	1.267	9.52	11.76	1.000	-0.243	0.198	0.789	0.00	0.00	1.000	0.000	0.000	0.000
rs62272114	A	G	0.00	0.00	1.000	0.000	0.000	0.000	4.76	0.00	1.000	-0.091	0.186	0.000	95.24	100.00	1.000	-0.186	0.091	0.000
rs77385406	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	17.65	0.182	-0.411	0.058	0.000	100.00	82.35	0.182	-0.058	0.411	0.000
rs61732118	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs61875109	A	C	14.29	5.88	0.746	-0.156	0.324	2.669	23.81	41.18	0.426	-0.523	0.176	0.446	61.90	52.94	0.821	-0.279	0.458	1.444
rs116369954	T	C	80.95	88.24	0.865	-0.353	0.208	0.566	19.05	11.76	0.865	-0.208	0.353	1.766	0.00	0.00	1.000	0.000	0.000	0.000
rs79805154	A	G	85.71	88.24	1.000	-0.265	0.214	0.799	14.29	11.76	1.000	-0.214	0.265	1.251	0.00	0.00	1.000	0.000	0.000	0.000
rs77064952	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs10059523	A	G	0.00	0.00	1.000	0.000	0.000	0.000	14.29	5.88	0.746	-0.156	0.324	2.669	85.71	94.12	0.746	-0.324	0.156	0.375
rs78183526	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs587667473	A	C	61.90	70.59	0.826	-0.440	0.266	0.677	33.33	29.41	1.000	-0.296	0.374	1.200	0.00	0.00	1.000	0.000	0.000	0.000
rs73179006	T	G	0.00	0.00	1.000	0.000	0.000	0.000	14.29	5.88	0.746	-0.156	0.324	2.669	85.71	94.12	0.746	-0.324	0.156	0.375
rs113262704	T	G	0.00	0.00	1.000	0.000	0.000	0.000	19.05	11.76	0.865	-0.208	0.353	1.766	80.95	88.24	0.865	-0.353	0.208	0.566
rs61989114	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.922	-0.224	0.106	0.000	100.00	94.12	0.922	-0.106	0.224	0.000
rs55656505	T	C	90.48	88.24	1.000	-0.198	0.243	1.267	9.52	11.76	1.000	-0.243	0.198	0.789	0.00	0.00	1.000	0.000	0.000	0.000
rs4783943	A	G	14.29	0.00	0.240	-0.060	0.346	0.000	38.10	58.82	0.335	-0.573	0.159	0.431	47.62	41.18	0.945	-0.306	0.434	1.299

Table 6.21 SNPs associated with TCF7L2

44 unique SNPs associated with TCF7L2 were identified, and genotyped. Following a two portions t-test significantly different prevalence's are highlighted

dbSNP RS ID	Minor allele (A)	Major allele (B)	% AA						% AB						% BB					
			Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio	Q1	Q4	*p*-value	95% CI		Odds Ratio
rs3026084	A	G	90.48	94.12	1.000	-0.241	0.168	0.594	9.52	5.88	1.000	-0.168	0.241	1.684	0.00	0.00	1.000	0.000	0.000	0.000
rs189314829	A	G	0.00	0.00	1.000	0.000	0.000	0.000	9.52	11.76	1.000	-0.243	0.198	0.789	90.48	88.24	1.000	-0.198	0.243	1.267
rs75107770	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	17.65	0.182	-0.411	0.058	0.000	100.00	82.35	0.182	-0.058	0.411	0.000
rs73223028	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.922	-0.224	0.106	0.000	100.00	94.12	0.922	-0.106	0.224	0.000
rs116929578	A	G	85.71	76.47	0.760	-0.212	0.397	1.846	9.52	17.65	0.803	-0.355	0.192	0.491	0.00	0.00	1.000	0.000	0.000	0.000
rs117041486	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.922	-0.224	0.106	0.000	100.00	94.12	0.922	-0.106	0.224	0.000
rs79565172	A	G	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs117582734	T	C	0.00	0.00	1.000	0.000	0.000	0.000	14.29	5.88	0.746	-0.156	0.324	2.669	85.71	94.12	0.746	-0.324	0.156	0.375
rs12243326	T	C	52.38	23.53	0.116	-0.058	0.635	3.575	38.10	52.94	0.554	-0.517	0.220	0.547	9.52	23.53	0.473	-0.431	0.151	0.342
rs4889830	A	G	33.33	35.29	1.000	-0.343	0.304	0.917	52.38	29.41	0.256	-0.128	0.587	2.640	14.29	35.29	0.259	-0.535	0.115	0.306
rs3787493	A	G	33.33	41.18	0.873	-0.440	0.284	0.714	38.10	41.18	1.000	-0.374	0.313	0.879	28.57	17.65	0.679	-0.209	0.427	1.866
rs4713505	T	G	9.52	5.88	1.000	-0.168	0.241	1.684	38.10	41.18	1.000	-0.374	0.313	0.879	52.38	52.94	1.000	-0.330	0.319	0.978
rs2070600	T	C	0.00	0.00	1.000	0.000	0.000	0.000	19.05	23.53	1.000	-0.352	0.262	0.765	80.95	76.47	1.000	-0.262	0.352	1.308
rs3131300	A	G	61.90	58.82	1.000	-0.313	0.374	1.137	28.57	23.53	1.000	-0.279	0.380	1.300	9.52	17.65	0.803	-0.355	0.192	0.491
rs73471190	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	5.88	0.922	-0.224	0.106	0.000	100.00	94.12	0.922	-0.106	0.224	0.000
rs148924158	A	G	100.00	100.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000
rs143828311	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs201967398	A	G	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs143652701	T	C	0.00	0.00	1.000	0.000	0.000	0.000	0.00	0.00	1.000	0.000	0.000	0.000	100.00	100.00	1.000	0.000	0.000	0.000
rs142236510	T	C	100.00	88.24	0.410	-0.089	0.324	0.000	0.00	11.76	0.410	-0.324	0.089	0.000	0.00	0.00	1.000	0.000	0.000	0.000

Table 6.21 SNPs associated with TCF7L2 (final)

44 unique SNPs associated with TCF7L2 were identified, and genotyped. Following a two portions t-test significantly different prevalence's are highlighted

Gene	dbSNP RS ID	A allele	B allele	SNP call rate (%)		MAF		H.W. P value		% AA			% AB			% BB		
				Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4	*p*-value	Q1	Q4	*p*-value	Q1	Q4	*p*-value
ABCC8	rs17743658	A	G	95.24	100.00	0.2500	0.3824	0.0369	0.1272	14.29	5.88	0.746	19.05	64.71	0.011	61.90	29.41	0.076
KCNJ11	rs506757	T	G	100	100	0.3810	0.4412	0.0581	0.0962	28.57	41.18	0.402	66.67	29.41	0.050	4.76	29.41	0.104
	rs13183249	A	G	100	100	0.1905	0.2941	0.0797	0.0858	9.52	0.00	0.564	19.05	58.82	0.029	71.43	41.18	0.126
HNF1a	rs663824	A	G	100.00	100.00	0.3333	0.3824	0.5127	0.0107	47.62	23.53	0.233	38.10	76.47	0.042	14.29	0.00	0.240
	rs2244608	A	G	100.00	100.00	0.1667	0.4118	0.3594	0.3770	66.67	29.41	0.050	33.33	58.82	0.214	0.00	11.76	0.377
	rs8102561	T	C	100.00	100.00	0.3571	0.1176	0.5190	0.5825	38.10	76.47	0.042	52.38	23.53	0.140	9.52	0.00	0.512

Table 6.22 Difference in prevalence of SNPs in SU+ diabetic cohort

A summary of SNPs that are significantly different between those receiving sulphonylureas after being divided into lowest (Q1) and highest (Q4) quartiles according to HbA1c.

6.4 Discussion

An important first point to make is that the results seen here are preliminary and should be treated with caution, while SNPs present have been associated with certain phenotypes in the literature, the work presented here is not robust enough to definitively state that this is the case in this cohort. Multiple tests have been performed on this data, and as always multiple testing itself can skew results seen, perhaps producing false positive associations. The results are also not wholly representative of the cohort as not all participants were sequenced due to a need to complete the project on time and within budget. More work is needed however, more detail of which is given later in this section.

In the current study, three SNPs were significantly associated with a T2DM phenotype. Several variants in KCNJ11 displayed significant differences in genotype between those with T2DM and control. rs78374474, associated with KCNJ11, is located on chromosome 8 at position 24469242. It lies in the gene RP11-624C23 (Type 2 Diabetes Knowledge Portal, 2017). The nucleotide at this position in the reference sequence is C, and the variant allele is T. Data available on the T2D knowledge portal suggests that while this variant is not significantly associated with a T2DM phenotype, there is a positive direction of effect that suggests a higher probability of having the disease. In our cohort, the prevalence of BB genotype of rs78374474 was significantly higher in those with T2DM compared with our control cohort.

Another variant associated with KCNJ11, rs76557252 is located on chromosome 13 at position 97201682, it lies within the gene HS6ST3 (Type 2 Diabetes Knowledge Portal, 2017). The nucleotide at this position in the reference sequence is G, and the variant allele is T. The data available for the SNP on the T2D knowledge portal is inconclusive for this variant, as the association with a T2DM phenotype is not significant and datasets

that show both a positive and negative direction of effect for probability of having the disease. The direction of effect may well depend on genotype. In this study, the AB genotype was less commonly observed in those with T2DM, while the BB genotype was significantly more prevalence in the T2DM cohort.

rs4713505 is associated with TCF7L2, and is located on chromosome 6 at position 32105001, it lies closest to the gene FKBPL (Type 2 Diabetes Knowledge Portal, 2017). The nucleotide at this position in the reference sequence is G, and the variant allele is T. In this study, the BB genotype was observed in the T2DM population at almost double the rate of the control cohort. Variation in TCF7L2 is among the strongest risk factors for development of T2DM (Peng et al., 2013). Evidence from the T2D knowledge portal has suggests rs4713505 is associated with numerous traits; of particular interest for comparison with results here is its association with T2DM phenotype.

The use of genetic variants to predict risk of developing a disease is useful particularly in populations that are known to be at risk (i.e. in the case of T2DM, the obese or prediabetic populations). However, a challenge that is common in T2DM is that while clinical features can predict disease well it is clear that predicting in other areas isn't as good, like predicting response to treatment. For those with an established diagnosis of T2DM, the potential use of genotyping to stratify drug treatment may have even greater utility and may help prevent adverse events and progression to many of the complications associated with long-term hyperglycaemia. This study has highlighted variants that also may be predictive of glycaemic control in the T2DM population, and those that appear to be associated with good glycaemic control in response to sulphonylurea treatment. For example, rs17743658 is associated with ABCC8, located on chromosome 18 at position 48476450 and lies within the gene ME2 (Type 2 Diabetes Knowledge Portal, 2017)

(a gene that is important for insulin secretion from pancreatic β-cells (Hasan et al., 2015)). The nucleotide at this position in the reference sequence is G, and the variant allele is A. In the current study the AB genotype was observed in those ranked in Q4 for HbA1c at almost twice the rate of those ranked in Q1. This suggests that the presence of this genotype may be associated with poor glycaemic control. Importantly, this was only observed in those on sulphonylureas. In the non-sulphonylurea groups, the difference between Q1 and Q4 was not significant.

rs13183249 is associated with KCNJ11 and is located on chromosome 5 at position 5807951, it lies closest to the gene ICE1 (Type 2 Diabetes Knowledge Portal, 2017). The nucleotide at this position in the reference sequence is G, and the variant allele is A. Another variant associated with the KCNJ11 gene in this study, rs663824, is located on chromosome 1 at position 43649508, It lies in the gene CFAP57 (cilia and flagella associated protein 57) (Type 2 Diabetes Knowledge Portal, 2017) and it significantly associated with aggressive prostate cancer (Chen, 2014). The nucleotide at this position in the reference sequence is A, and the variant allele is G. Little is known about these variants in relation to T2DM risk or glycaemic control. Prevalence of the AB genotype of each of these variants had differing association with glycaemic control in the current study. rs13183249 was more prevalent in Q1, whilst rs663824 was more prevalent in Q4, reflective of the fact that variants can be both protective and detrimental when it comes to glycaemic control. Again, it is important to note, that these observations were exclusive to those on sulphonylureas in this study. Participants receiving other anti-diabetic drugs did not show differential expression of any genotype associated with these SNPs. rs506757 is associated with KCNJ11, and is located on chromosome 13 at position 97303709, it lies in the gene HS6ST3 (Type 2 Diabetes Knowledge Portal, 2017), a gene that has been linked to the progression of T2DM (Li et al., 2016). The nucleotide at this position in the

reference sequence is T, and the variant allele is G. There is a positive direction of effect for its association with a T2DM phenotype, and HbA1c according to data from the T2D Knowledge portal.

The variant rs2244608 is located on chromosome 12 at position 121416988; it lies in the gene HNF1α itself (Type 2 Diabetes Knowledge Portal, 2017). The nucleotide at this position in the reference sequence is A, and the variant allele is G. This particular polymorphism has been cited for its association with C-reactive protein (Reiner et al., 2008), and data from the T2D Knowledge portal suggest a significant association with a T2DM phenotype and a negative direction of effect for its association with HbA1c. Consistent with this, an association between the AB genotype and good glycaemic control was observed here. Finally, rs8102561 associated in this study with the HNF1α gene, is a variant located on chromosome 19 at position 55488150, it lies in the gene NLRP2 (Type 2 Diabetes Knowledge Portal, 2017), which inhibits the NF-kappa B signaling pathway and regulates Caspase-1 (Bruey et al., 2004, Fontalba et al., 2007). The nucleotide at this position in the reference sequence is T, and the variant allele is C. From data available on the T2D knowledge portal, there is no significant association with a T2DM phenotype and data sets have both a positive and negative direction of effect for probability of having the disease. However, in the current study, the AB genotype was again found to be significantly associated with poor glycaemic control, only in those receiving sulphonylureas.

This work is a preliminary analysis and further refining is needed. DNA from the remainder of the cohort needs to be sequenced. The current analysis is also limited by population size. Recruitment is ongoing to increase the number of control participants to a minimum of 200, which will have to be sequenced also. Reanalysis of the data at this stage will validate the findings of this study. Furthermore, the findings require validation in secondary cohorts of individuals with T2DM and controls

and collaborations are being established with centers throughout the UK to allow this to happen. The computational team at the NI Centre for Stratified Medicine are also undertaking further analysis of the current dataset to interrogate the relationship between genotype and other T2DM-associated phenotypes including BMI, c-peptide and lipid profiles. At that point in the future, when this further work has done, further more sophisticated statistical analysis can take place, making the results seen more robust.

Nonetheless, the current work suggests that there is a unique panel of SNPs associated with a T2DM phenotype and with poor glycaemic control in this population. Of particular interest and worthy of further investigation, this study has revealed several SNPs within ABCC8, KCNJ11 and HNF1α that appear to be associated with good glycaemic control in those receiving sulphonylurea therapy, but not in those on other classes of anti-diabetic drugs. Although this finding requires further validation, it is an exciting discovery and may be useful in assessing response to the sulphonylurea drug class, which is associated with poor response rates and a significant adverse event profile (Aquilante, 2010).

Chapter 7:

General Discussion

7.1 Heterogeneity of the diabetic population

Type 2 diabetes mellitus (T2DM) is a multifaceted disease, which arises through a clash of genetic and environmental factors, and which is reaching epidemic proportions globally (Danaei et al., 2011, Alberti and Zimmet, 2014, Zimmet et al., 2014). It is a complicated heterogeneous collection of metabolic disorders that includes hyperglycaemia and impaired insulin secretion and/or action (Lin and Sun, 2010). There is increasing evidence to show that diabetes is far more heterogeneous than previously thought. Recent data suggest that merely grouping those suffering with this chronic disease as type 1 or type 2 is simplifying the broad spectrum of factors at play in the disease, and likely means there are far more subgroups prevalent in the increasing numbers of those diagnosed (Tuomi et al., 2014). For example, β-cell autoantibodies were identified in a subgroup of children and adolescents who presented clinically as having T2DM (Reinehr et al., 2006). Furthermore, topological analysis of patient similarities revealed that people with T2DM can be classified into three distinct groups that predict the development of specific diabetic complications (Li et al., 2015).

Given this, the approach to treating T2DM is notoriously complicated with the NICE care pathway offering multiple stages of assessment and intervention. Stratified or Precision Medicine has offered the hope that much of the trial and error approach to the treatment of T2DM may be solved by identifying specific biomarkers or drivers of glycaemic control. A great deal of research has focused on the identification of genetic biomarkers. However, clinical parameters and indeed proteomic or metabolomics markers may also be of significant clinical utility. To this end, the current study sought to characterize clinical, proteomic and genetic markers associated with glycaemic control in the DIASTRAT cohort, with particular emphasis on analysis of those receiving sulphonylurea therapy, which is an antidiabetic drug class associated

with poor response rates and significant adverse events (Aquilante, 2010).

In the current study, the DIASTRAT cohort were recruited solely from the Western Health and Social Care Trust, which covers an area of 4,842 sq. km, taking in counties Derry, Tyrone and Fermanagh. Participants were recruited from within this region only, with the vast majority being recruited from Derry City and surrounding areas, and participants were relatively homogenous in terms of ethnicity. This cohort was almost exclusively recruited from secondary care clinics, which suggests an advanced stage of disease, but may also raise additional complications of comorbidities. Within the DIASTRAT cohort 51% of participants were found to have a clinical diagnosis for 5 or more comorbid conditions. The association of T2DM and comorbid conditions, and the subsequent risk of complication, has been widely reported (Hollander and Kushner, 2010, Kim et al., 2012, Pantalone et al., 2015)

The incidence of T2DM increases with age and the condition disproportionately affects people aged 65 or older. Consistent with this, the mean age of the DIASTRAT cohort was 63. The advancing age of the T2DM population and the diagnosed comorbidities discussed above, increases the likelihood, and indeed occurrence, of polypharmacy (Noale et al., 2016). It is typical that older people are more likely to suffer from chronic medical conditions like hypertension, obesity, or dyslipidemia that leads to the need for additional drugs (Noale et al., 2016). In the DIASTRAT study, 14% of participants were on 4 or more antidiabetic drugs. However, the scale of the polypharmacy within this group is better understood when non-antidiabetic treatments are considered. There are some 69% of participants receiving at least 5 treatments, and 17% of participants receiving at least 10 treatments.

The current study has identified several factors of relevance to clinical decision making: Firstly, the concomitant prescription of a sulphonylurea with an exogenous insulin preparation was associated with higher BMI and worsened glycaemic control when compared with those receiving either drug in isolation. The co-prescribing of these drugs is not an isolated phenomenon in our cohort. Recent work in a Canadian cohort of 458 people with T2DM suggested that 18% were on both insulin and sulphonylureas for more than one year (Ratzki-Leewing et al., 2017). Given that both prescriptions seek to restore circulating insulin levels, it appears contradictory to permit long-term administration of both drugs in combination. Secondly, the data reveal poor glycaemic control among the DIASTRAT cohort, which is countered by excellent lipid profiles. Current NICE guidelines are heavily focused on the control of cholesterol to prevent the development of cardiovascular complications (National Institute for Health and Care Excellence (NICE), 2015). These targets are clearly being met within the DIASTRAT cohort, but this is potentially at the expense of glycaemic control, which will elevate the risk of macrovascular complications independently of cholesterol levels. Consistent with this, peripheral protein markers in the DIASTRAT cohort were heavily associated with Coronary Artery Disease.

7.2 Proteomic markers of glycaemic control

There is a dearth of potential proteomic markers of glycaemic control. The key role played by inflammatory processes in diabetes (Wellen and Hotamisligil, 2005, Alexandraki et al., 2006, Montane et al., 2014) and in obesity-driven insulin resistance (de Luca and Olefsky, 2008, Monteiro and Azevedo, 2010, Cavalcante-Silva et al., 2015) is well studied and reported. With that in mind, the current study sought to identify protein markers of glycaemic control within the DIASTRAT cohort.

The results identified an initial 75-protein signature that distinguished those with T2DM from apparently healthy controls with 99.2% accuracy. While this is indeed interesting, it is not necessarily clinically significant as we can already identify those with T2DM via less expensive glucose monitoring or biochemical tests. However, the identification of a unique protein signature did reveal several important factors about the inflammatory processes in this cohort. Firstly, inflammation is macrophage driven, consistent with findings that macrophages are present in adipose tissue and that obesity can alter the activation status of these cells (Donath and Shoelson, 2011). Obesity can promote a chronic low-grade inflammation, which is related to parainflammation (Medzhitov, 2008). Parainflammation is crucial to the interaction between metabolism and inflammation. The dysregulation of this inflammation is linked to insulin resistance, through the increase of cytokines like tumor necrosis factor-α (TNF-α), and macrophage infiltration into adipose tissue (Thomas and Apovian, 2017). There are a plethora of outcomes to this dysregulation, including chronic systemic inflammation, metabolic syndrome, obesity-related insulin resistance, non-alcoholic fatty liver disease and, importantly for this study, T2DM (Nikolajczyk et al., 2012, Thomas and Apovian, 2017). Also in this cohort CD4+ T cells did not have a primary role to play in driving the inflammatory process, contrary to current evidence (Shu et al., 2012, Xia et al., 2017). Consistently, protein markers poorly expressed in the DIASTRAT cohort were heavily associated with CD4+ T cells. Few of those identified have previously been associated with disease pathogenesis. Perhaps not surprisingly, a single protein marker for glycaemic control could not be identified within this cohort – the volume of inflammatory, genetic and environmental factors involved in the genesis and progression of this disease meant that this was always likely to be the case. However, the work was not without merit. For example, proteins that were identified within the DIASTRAT cohort, KIM1 FGF23, FGF21, were each associated with elevated HbA1c. A finding that is hoped can be further validated in larger cohorts in the future. In

addition, many of the markers identified here have been implicated in development of secondary complications. Therefore, there may be significant clinical benefit in screening for these markers in the prediction, or even prevention, of many of the devastating complications of the disease.

7.3 Genetic variants associated with T2DM and glycaemic control

The contribution and effect of genetic variants in the development of T2DM is wide and varied. The current study sought to investigate the role of specific genes previously identified as important in sulphonylurea response at a single cell level in the pancreatic β-cell and globally, within populations.

The work presented in this thesis confirms a role for both ABCC8 and KCNJ11 in the response to sulphonylurea treatment. This is largely unsurprising given that these genes encode the protein subunits of the K_{ATP} channel, the primary target of the sulphonylurea drug class. From a mechanistic perspective, silencing of either ABCC8 or KCNJ11 hampered sulphonylurea-potentiated insulin secretion from β-cell lines. Consistently, several SNPs associated with ABCC8 and KCNJ11 were found to be significantly associated with glycaemic control in those receiving sulphonylureas, but not in those receiving other anti-diabetic therapies. These results are consistent with work produced by other groups (Meirhaeghe et al., 2001, Zychma et al., 2002, Schroner et al., 2011, Ragia et al., 2012, Li et al., 2014, Song et al., 2017), but it should still be noted that is contradicted by results in other work also (Haghvirdizadeh et al., 2015). Going forward, it will be important to validate the direct mechanistic impact of these SNPs on insulin release via CRISPR/Cas9 studies in isolated β-cell lines and primary islet cells.

The association between risk of T2DM, or sulphonylurea response, and certain variants has been studied in the past. Variants for each of the genes of interest within this work have been highlighted, thus providing extra validation for pursuing the prevalence of polymorphisms within these genes within the DIASTRAT cohort, and then to define what association existed with glycaemic control, particularly in those who were prescribed sulphonylureas. There are over 80 common variants for T2DM that have already been identified, but which generate a small effect, with a minor allele frequency >5%. These variants therefore account only for around 10% of heritability of T2DM (Stancakova and Laakso, 2016, Fuchsberger et al., 2016). The results for the DIASTRAT cohort showed variants are potentially predictive of glycaemic control or are associated with response to sulphonylureas. In an attempt to corroborate the conclusions drawn from these results, publically available datasets held within the T2D Knowledge Portal (http://type2diabetesgenetics.org/home/portalHome) were consulted. This work confirmed that several variants identified from the current analysis are associated with a variety of traits consistent with a T2DM phenotype. Despite the limited sample size, the current work suggests that a unique panel of SNPs associated with a T2DM phenotype and with poor glycaemic control exists in this population.

7.4 Concluding remarks: integration of clinical and molecular data for prediction of glycaemic control in T2DM.

The aims of this work were four-fold: (1) Identify and validate existing markers of sulphonylurea response in two pancreatic β-cell lines; (2) Establish a biobank of 500 T2DM patients (DIASTRAT cohort) with associated clinical, anthropometric, and biochemical data; (3) Conduct proteomic screens for novel inflammatory or metabolic markers

associated with glycaemic control and sulphonylurea response in the DIASTRAT cohort; and (4) assess the prevalence of potentially important SNPs among the DIASTRAT cohort and assess the relationship with glycaemic control and sulphonylurea response.

To date, T2DM research has tended to focus on either genetic or clinical markers of glycaemic control in isolation. However, the use of simple clinical information can also provide significant benefit when making clinical decisions. Shields et al. (2016) showed that BMI and sex should be considered when selecting a sulphonylurea. Their results pointed to better glycaemic response in non-obese males when compared to obese females. This example suggests that clinical data may also help to stratify patients. The integration of clinical, proteomic and genomic information expands the potential for positive patient outcomes and cost savings for health services.

The results shown for DIASTRAT do come with caveats, most notable of which is the small sample size, which restricts the conclusions that can be drawn from the work. However, work is ongoing to expand the project into other centres in the UK and validate the results seen here in larger secondary cohorts. The observations here that clinical parameters, proteomic and genetic markers are differentially expressed in those with and without T2DM further reflect the heterogeneity of the disease. The current study has identified unique protein and genetic markers that are associated with a T2DM phenotype and further identified several novel targets for sulphonylurea response. Pending further validation, it is hoped that these findings may prove of clinical utility in the treatment of T2DM. Furthermore, the work highlights the importance of considering clinical, proteomic and genetic parameters to this end.

Chapter 8:

References

References

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